THE ROLE OF OMEGA-6 TO OMEGA-3 FATTY ACID RATIOS IN SOW DIETS ON REPRODUCTION, PIGLET PERFORMANCE, FATTY ACID PROFILES, LACTATIONAL FAT MOBILIZATION AND PIGLET HEALTH POST-WEANING

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ABSTRACT

A series of experiments was conducted to test the overall hypothesis that reducing the omega-6 (n-6) to omega-3 (n-3) fatty acid (FA) ratio in sow diets would improve sow reproductive performance (characterized by increases in numbers and body weight of piglets born alive and weaned) and would lessen the inflammatory responses of their offspring post weaning. Diets were wheat/barley based and consisted of a control (tallow based, similar to a standard production diet), 3 diets with plant oil based n-6:n-3 ratios (9:1P, 5:1P, and 1:1P) and a 5:1 fish oil diet (5:1F). The control diet had a ratio of 8:1, but contained approximately half the polyunsaturated FA content of the other diets. Sows were randomly assigned to a treatment diet on d 80 of gestation, and remained on that treatment for three consecutive reproductive cycles (gestation/lactation 1 = P1, gestation/lactation 2 = P2, gestation/lactation 3 = P3).

Experiment 1 was designed to test the hypothesis that reducing the n-6:n-3 FA ratio in sow diets would increase circulating concentrations of n-3 FA's in sows and in their offspring, and the passive immune status of piglets would be improved. Performance data was collected throughout P1 and P2 on 150 sows (n = 30/diet). Sow and piglet serum, colostrum and milk were analyzed for FA profiles, and colostrum and piglet serum were analyzed for immunoglobulin (Ig) A and IgG. In P1, birth weights were unaffected by diet ($P > 0.05$). Average piglet weaning weights ($P = 0.02$) and ADG ($P = 0.01$) however, were highest for piglets born to sows consuming the 9:1P and 5:1P diets. During P2, 5:1F sows consumed 10% less feed ($P = 0.04$), their piglets had reduced birth weights ($P = 0.05$), and average weaning weight was reduced by 0.8 kg ($P = 0.04$) relative to control or 5:1P sows. Colostral and piglet plasma IgA and IgG were unaffected by diet ($P > 0.05$). Colostrum FA profile patterns were similar to that of the sow diets. Serum n-3 FA's were greatest in sows ($P < 0.01$) and piglets ($P < 0.01$) consuming 1:1P or 5:1F diets. Serum α-linolenic acid (ALA) was highest in the 1:1P sows and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were highest in the 5:1F sows. In piglet serum obtained prior to suckling, ALA and DHA did not differ among treatments ($P > 0.05$) but EPA was 2.5 times greater in the 1:1P group and 4 times greater in the fish group ($P < 0.01$) compared to those from the control diet. In post-suckle samples, ALA was highest in serum from 1:1P diet piglets ($P < 0.01$), and EPA and DHA were highest in piglet serum from the 5:1F sows ($P < 0.01$).

Omega-3 FA's can perturb lipid metabolism, specifically increasing the lipolytic activity of adipose tissue and thus the second experiment tested the hypothesis that high producing sows,

consuming reduced n-6:n-3 ratios would have increased body fat mobilization. Twenty sows per diet, farrowing ≥ 11 piglets and nursing ≥ 10 piglets during P3, were used. Performance data on sows and piglets (such as weights, numbers, backfat changes) was collected throughout lactation and milk samples obtained on d 4 and d 16 of lactation. Jugular catheters were inserted into 8 sows from each of the 9:1P and 1:1P groups on d 5 of lactation and sows were challenged with a single injection of epinephrine followed by serial blood collections. Feed intake was highest for sows consuming the control (8.4 kg/d) and 5:1P (8.2 kg/d) diets and lowest for the sows fed the 1:1P (7.4 kg/d) and 5:1F (7.7 kg/d) diets ($P = 0.05$). Altering the n-6:n-3 FA ratio did not affect sow BW, piglet ADG, milk DM and N content or the total output of milk ($P > 0.2$). Sows consuming the 1:1P diet had greater backfat thickness ($P < 0.05$) and numerically higher plasma NEFA at baseline compared with the 9:1P sows (240 vs 93 uM; $P = 0.16$). When given epinephrine, 9:1P fed sows tended to have lower net incremental area under the curve (niAUC) glucose ($P = 0.08$) and numerically higher niAUC NEFA ($P = 0.17$) and glycerol ($P = 0.15$).

A third experiment was conducted to test the hypothesis that piglets raised by sows consuming reduced n-6:n-3 ratios would have reduced inflammatory responses post-weaning. Piglets (n = 20/diet) raised by sows consuming the treatment diets described above for 2 gestation/lactation cycles (P2) were selected at weaning. Within diet group, pigs were randomized to either a challenge control group (saline injected) or to a lipopolysaccharide (LPS) injected group ($n=10/\text{challenge}\cdot\text{diet}^{-1}$). Piglets were fed a common starter diet for 6 days followed by saline or LPS injections on d 7. Rectal temperatures were recorded for 24 hrs and blood samples were collected at 0, 2, 6 and 12 hrs post injection for pro-inflammatory cytokine and blood urea nitrogen (BUN) analysis. Injecting LPS caused decreased feed intake and reduced ADG ($P < 0.01$), and increased temperature and cytokine production ($P < 0.05$). Piglets raised by sows consuming the 1:1P diet had elevated temperatures ($P = 0.01$; diet x challenge $P > 0.05$).

Overall, circulating plasma ALA and EPA were increased in sows and piglets when sows were fed a 1:1 plant based ratio compared to the control or high n-6:n-3 ratio groups. Sows fed a ratio of 1:1 mobilized more body fat relative to those consuming the 9:1 ratio; there were no treatment effects on piglet growth. Reducing maternal n-6:n-3 FA ratios below 5:1 increased piglet body temperature prior to and during an LPS induced inflammatory challenge,. Reducing the sow dietary n-6:n-3 FA ratio below 5:1 may have detrimental effects on piglets due to over-stimulation of inflammatory responses.

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DEDICATION

I would like to dedicate this thesis to my family. To my parents, Elaine and Steve, I am grateful to have such wonderful, loving parents; my life wouldn't be what it is today without you. You have made me who I am today, and I never would have believed I could achieve so much without your support. To my grandmother Freda, your continual love, encouragement and support means the world to me. To my siblings Nicky and Mark and your wonderful families, I don't know what I would do without you; you are always there to support me and to give me a laugh when I need it. I love you all very much, and couldn't be more grateful to be a part of such a wonderful family.

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LIST OF ABBREVIATIONS

1:1P	1:1 omega-6 to omega-3 ratio, plant based diet
5:1F	5:1 omega-6 to omega-3 ratio, fish based diet
5:1P	5:1 omega-6 to omega-3 ratio, plant based diet
9:1P	9:1 omega-6 to omega-3 ratio, plant based diet
ArA	Arachidonic acid (C20:4, n-6)
ADFI	Average daily feed intake
ADG	Average daily gain
AIC	Akaike information criteria
ALA	α-linolenic acid (C18:3, n-3)
ALAin	Dietary α-linolenic acid intake
AUC	Area under the curve
BIC	Bayesian information criteria
DHA	Docosahexaenoic acid (C22:6, n-3)
DM	Dry matter
DM_L	Milk dry matter production (g per piglet per day)
E_L	Milk energy production (kcal per piglet per day)
EPA	Eicosapentaenoic acid (C20:5, n-3)
ETA	Eicosatrienoic acid (C20:4, n-3)
FA	Fatty acid
FAME	Fatty acid methyl ester
FSM	Flaxseed meal
FSO	Flaxseed oil
Glu	Glucose
Ig	Immunoglobulin
IL	Interleukin
HPA	Hypothalamic pituitary adrenal axis

LA	Linoleic acid (C18:2, n-6)
LC	Long chain (> 20 carbon) fatty acids
LPS	Lipopolysaccharide
M	Milk production (g per piglet per day)
MUFA	Monounsaturated fatty acid
N	Nitrogen
N_L	Milk nitrogen production (g per piglet per day)
n-3	Omega-3 fatty acid
n-6	Omega-6 fatty acid
n-6:n-3	Omega-6 to omega-3 fatty acid ratio
n-9	Omega-9 fatty acid
NEFA	Non-esterified fatty acid
niAUC	Net incremental area under the curve
P1	Experimental time period 1, d80 of gestation to weaning
P2	Experimental time period 2, breeding to weaning subsequent to P1
P3	Experimental time period 3, breeding to weaning subsequent to P2
PG	Prostaglandin
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PSY	Pigs per sow per year
PUFA	Polyunsaturated fatty acid
PVC	Polyvinyl chloride
sEPA	Serum eicosapentaenoic acid content
sEPA:ALAin	Serum eicosapentaenoic acid content to dietary α-linoleic acid ratio
sLC	Serum long chain fatty acid content (sum of ETA, EPA and DHA)
sLC:ALAin	Serum long chain fatty acid to dietary α-linolenic acid ratio
TLR	Toll like receptor
TNF-α	Tumor necrosis factor α

1 GENERAL INTRODUCTION

In swine production, the breeding and farrowing to weaning periods are critical. Conception rates, farrowing rates and piglet survival during lactation affect pig flow throughout the entire facility and system. Modern sows are prolific, and due to continual improvements in genetics and management, litter sizes have increased dramatically (PigChamp, 2011). The number of piglets weaned per sow lifetime however, has not improved at the same rate. As the total number of pigs born increases, the number born alive stays relatively constant, or the birth rates of small or weak piglets with low survivability increases (Boulot et al., 2008). Additionally, as piglet numbers increase there is an increasing energy demand on the sow for nutrient output in the milk, and if these nutrients are not obtained from the diet, body reserves will be mobilized, with potential negative impacts on subsequent reproductive cycles (Tantasuparuk et al., 2001).

There have been many nutritional strategies implemented with the aim of improving reproductive performance of sows and improving piglet survivability. Recently, there has been a growing interest in the pork industry for the use of dietary long chain polyunsaturated fatty acids (PUFA's), due to the fact that PUFA's have been implicated as having many potential health benefits.

Polyunsaturated fatty acids are precursors for many different hormones and molecules (Lands, 1992). Linoleic acid (LA) and α-linolenic acid (ALA) are precursors for the eicosanoids, which includes prostaglandins (some of the most abundant molecules found in the body), leukotrienes and thromboxanes (Lands, 1992). Omega-6 (n-6) fatty acids (FA) such as LA are precursors for one set of eicosanoids (2-series prostaglandins, 4-series leukotrienes and 3-series thromboxanes) while the omega-3 (n-3) FA's such as ALA are precursors for a different set of eicosanoids (3-series prostaglandins, 5-series leukotrienes and 3-series thromboxanes). In general, the molecules produced by the n-6 FA's are considered to have greater biological activity than those formed by the n-3 FA's (Palmquist, 2009).

Within the *de novo* synthesis pathway there is direct competition for the desaturation and elongation enzymes between the n-6 and n-3 FA's (Lands, 1992). Formation of the 20 carbon FA's from their 18 carbon precursors requires the action of

delta-5 and delta-6 desaturase enzymes (Sprecher, 2000). Although these enzymes have a greater affinity for the n-3 FA's (Palmquist, 2009), typical western diets, especially corn/soybean meal diets (and to a lesser degree wheat/barley based diets), contain 15-20 times more n-6, leading to greater production of the n-6 derived eicosanoids (Simopoulos, 1991). Theoretically, reducing the dietary n-6 to n-3 FA ratio should increase the production of n-3 based eicosanoids at the expense of the n-6's. As discussed below, n-3 FA's have potential benefits on immunity and reproduction, and thus reducing the dietary n-6 to n-3 ratio may improve animal health, performance and reproduction.

Much of the scientific literature discusses n-3 FA's in two groups, the less biologically active ALA; versus the longer chain, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which are believed to be responsible for many of the health and reproductive benefits attributed to n-3's. Both DHA and EPA are obtained from either direct consumption (marine based sources such as fish oil), or synthesized in the body utilizing dietary ALA as the precursor (Voet et al., 2008). As mentioned above, synthesis of the long chain n-6 and n-3 FA's from their shorter precursors is a competitive process as they utilize the same enzymes (Lands, 1992). Estimates of the efficiency of the conversion of ALA into its longer chain counterparts in humans are variable (Brenna et al., 2009; Burdge and Calder, 2005; Goyens et al., 2006; Welch et al., 2008); however, it is apparent that this conversion is not a very efficient process (<10-15%). Within the swine literature, there are few estimates of this conversion. However, the conversion rate in pigs appears to be more efficient than in humans based on estimates using a serial slaughter trial and mathematical modeling (Martinez-Ramirez et al., 2008). Importantly, studies looking at the conversion of ALA into EPA and DHA have shown that the conversion is dependent on the ratio of the FA's in the diet, not the absolute amount (Harnack et al., 2009).

There are several review papers which discuss the effects of n-3 FA's on reproduction in animals and humans (Abayasekara and Wathes, 1999; Allen and Harris, 2001; Wathes et al., 2007). A series of studies has shown that alteration of dietary PUFA content in cattle alters the number and size of pre-ovulatory follicles, ovulation rate, conception rate, progesterone production by the corpus luteum and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) production which in turn affects luteolysis and gestation length (Petit et al.,

2001; Petit et al., 2002; Petit and Twagiramungu, 2006). In pigs, inclusion of dietary n-3 FA's into sow diets increased the PUFA content in sow and piglet plasma and in milk (Fritsche et al., 1993; Rooke et al., 2001b); which has been attributed to improving pre-weaning survival in some studies, but not others. Spencer et al. (2004) found an increase in the number of piglets born alive when sows were fed a source of fish oil (high in n-3).

A study by Dunstan et al. (2004) found that women fed fish oil had increased IgA concentrations in their milk. Additionally, Jackson et al. (1995) showed that sows fed corn (high n-6 FA's) had depressed IgG concentrations in their milk. A recent article by Mateo et al. (2009) reported increased IgG concentrations in the colostrum and milk of sows fed a fish product. The Ig's are large glycoproteins transferred to the piglet through colostrum and milk which provide antibody protection (Tizard, 2009). Immunoglobulin G plays a major role in antibody mediated defence mechanisms, and assists in the inflammation process (Tizard, 2009).

As well as influencing the eicosanoids, there is evidence that intake of n-3 FA's alters the production of cytokine molecules (Simopoulos, 2002). Cytokines are proteins which are secreted by immune cells in response to stimuli, and assist in regulating the development of immune effector cells or act as effectors themselves (Tizard, 2009). It is believed that n-3 FA's modulate cytokine function by acting upon intracellular signalling pathways, transcription factor activity and gene expression (Simopoulos, 2002). Essentially, interactions between immune and inflammatory cells are mediated by the inflammatory cytokines, of which tumour necrosis factor (TNF-α), interleukin (IL)-1, IL-6 and IL-8 are important (Tizard, 2009). They are produced by monocytes and macrophages, and production of appropriate amounts in response to infection is important and beneficial; however, over or inappropriate production of these molecules can be detrimental. Generation of an appropriate immune response is essential when an animal is immunologically challenged but it has been well documented that the pro-inflammatory cytokines, when produced in excess, increase muscle degradation, reduce protein synthesis and divert nutrients for the synthesis of immune molecules (Zhan et al., 2009). Since eicosanoids are formed from n-3 and n-6 FA's, and those produced by n-3 FA's are anti-inflammatory, it would be valuable to explore whether alteration of dietary n-6 to n-3 FA ratios for sows could improve piglet health.

Sow and piglet performance are affected by the sow's ability to produce milk, and the amount and composition of dietary fat may play a role in milk output. Lactational homeorhesis is defined as "the physiological drive to produce milk at the expense of other body functions" (Pettigrew et al., 1993). This phenomenon describes the metabolic state of the lactating sow who experiences a period of negative energy balance and adipose tissue mobilization to support milk production (Pettigrew et al., 1993). Hypophagia is commonly observed immediately following farrowing and contributes to the inability of sows to meet nutrient demands for maximal milk production (Weldon et al., 1994a, b). Although milk production increases in response to litter size (Auldist et al., 1998) the extent of this is unknown and milk production is probably a limitation to optimal growth in the larger litters found in modern genotypes (Boulot et al., 2008). Hormonal regulation of appetite and milk production may affect the degree of negative energy balance encountered by modern sows.

The importance of adipose tissue in the maintenance of lactation, especially during early lactation, has been extensively researched in the high-producing dairy cow (McNamara, 1991, 1989; Patton et al., 2007). It is important to determine if the lactating sow responds similarly to the cow and thus facilitate extrapolation of the large amount of data available. In the cow, a dramatic increase in catabolism and decrease in anabolism occurs in adipose tissue shortly after parturition (McNamara, 1991). This is partly a whole-body response to the negative energy balance resulting from the energy output in milk and decreased feed intake around parturition. However, experiments conducted on adipose tissue *in vitro* demonstrate that the adipose tissue responsiveness is also altered (McNamara and Hillers, 1986). It has been shown in several studies that the FA profile of adipocytes reflects the composition of dietary lipids (Eastwood et al., 2009; Fickova et al., 1998) and furthermore that replacement of n-6 FA's with FA's of the n-3 series has profound effects on reducing plasma triglycerides (Fickova et al., 1998). The effect of n-3 FA's on lipogenesis and lipolysis is less clear and inconsistent across studies, and thus the role of body fat composition is not well understood in terms of whole body metabolism during lactation.

The effects of altering the dietary FA ratio (n-6 to n-3) on sow reproductive efficiency, milk production, piglet performance, and piglet health have not yet been

determined. It has been hypothesized that the ratio of the FA's is more important than the absolute amounts for many biological functions. Determining how the ratio will affect the reproductive lifespan of a sow and improve her production throughout that life will aid producers in maximizing profits and improve sow management practices. Therefore, the following series of experiments were designed to look at the effects of altering the dietary n-6 to n-3 ratio for sows and to determine the differences between plant based sources of n-3 (ALA) and fish based sources (DHA and EPA) in terms of animal performance, metabolism and health.

2 LITERATURE REVIEW

2.1 The Modern Sow

The modern sow is a prolific animal (Kim et al., 2007), producing an average of 25-30 pigs per sow per year (PSY), or 12 piglets born alive per litter (PigChamp, 2011). In order for a sow to be profitable, she must be productive for 3 or more parities (Stalder et al., 2003), and must wean strong, healthy piglets. Over the last 15 to 20 years, genetic (Peet, 2008), nutritional and management (Boulot et al., 2008) improvements have allowed for dramatic increases in sow reproductive performance. However, the development of prolific sows has also been associated with increases in preweaning mortality, reduced piglet growth and increases in the numbers of small, weak and immune-compromised piglets (Boulot et al., 2008; Foxcroft, 2008).

Nutritional management of the modern sow is a key aspect of ensuring that she is able to produce to her maximal capacity and remain profitable for the producer. The nutrition of the sow directly affects fetal and neonatal development, growth and piglet health (Kim et al., 2007); and also impacts her reproductive lifespan, health and thus herd longevity. Although many nutritional strategies have been implemented, there is still room to develop new feeding strategies aimed at benefiting overall herd performance and animal health, as piglet mortality remains high and early culling of sows limits profitability within the pork industry.

2.2 Polyunsaturated Fatty Acids

Polyunsaturated FA's are long chain FA's (≥18 carbons) that contain more than one double bond. They are classified as omega-3 (n-3), omega-6 (n-6), omega-9 (n-9) or conjugated FA's based on the placement of the double bonds (Gurr et al., 2002). Most commonly, the numbering of a FA chain is done from the carboxyl end of the molecule, with the carboxyl carbon being C1; however, the omega terminology (n-3, n-6, n-9)

refers to the position of the first double bond relative to the methyl end of the molecule (Gurr et al., 2002). The double bonds in a FA chain can be either a *cis* or *trans* bond, and can be either methylene interrupted (separated by a methylene group) or conjugated. Most PUFA's found in mammals and plants contain methylene interrupted, *cis* configuration double bonds (Gurr et al., 2002).

Polyunsaturated FA's are derivatives of monounsaturated fatty acids (MUFA), where the first double bond typically occurs at the Δ^9 position (9^{th} carbon from the carboxyl end of the molecule). Palmitate (C16:0), is the shortest of the FA's which can be elongated and potentially desaturated into longer chain saturated or unsaturated FA's. This is done through the action of elongase and desaturase enzymes (Voet et al., 2008). Elongases are present in mitochondria and the endoplasmic reticulum (Gurr et al., 2002). In the mitochondria, elongation occurs by the successive addition and reduction of acetyl units in a reversal of FA oxidation (Gurr et al., 2002). Elongation in the endoplasmic reticulum involves the successive condensations of malonyl-CoA with acyl-CoA (Horton et al., 2002).

Unsaturated FA's are produced by terminal desaturases (Gurr et al., 2002). Mammalian systems contain four terminal desaturases of broad chain-length specificities designated Δ^9-, Δ^6-, Δ^5- and Δ^4-fatty acyl CoA desaturases (Gurr et al., 2002). Double bonds are inserted between existing double bonds such that the new double bond is three carbons closer to the CoA group than the next (separated by a methyl group). In animals, there is never a double bond beyond position C9 (Voet et al., 2008). The Δ^6-desaturase step is the rate limiting step in the desaturation and elongation pathway (Gurr et al., 2002).

A variety of unsaturated FA's can be synthesized by combinations of elongation and desaturation reactions. The most important precursors for polyunsaturation are oleic acid (C18:1, n-9), linoleic acid (C18-2, n-6) and α-linolenic acid (C18:3, n-3) (Voet et al., 2008). Oleic acid has a single double bond at the Δ^9 position, and can be synthesized by the animal or consumed in the diet (Horton et al., 2002). Linoleic and α-linolenic acid have multiple double bonds, including bonds located at the Δ^{12} and Δ^{15} positions. These FA's cannot be synthesized in the body as mammals lack Δ^{12} and Δ^{15} desaturase enzymes. They must be consumed and are therefore considered essential (Gurr et al.,

2002). The longer chain derivatives of these 18 carbon FA's can then be formed through elongation and desaturation pathways.

2.2.1 Functions in the Body

Polyunsaturated FA's are essential for many biological pathways, and they have significant roles in growth, health and reproduction (Gurr et al., 2002). As mentioned above, the n-6 and n-3 FA's cannot be synthesized in the body, and therefore must be consumed. Linoleic acid (the primary n-6) and α-linolenic acid (the primary n-3) are the 18 carbon precursors for the longer chain arachidonic acid (ArA; n-6), EPA (n-3) and DHA (n-3). The 20 carbon ArA and EPA are precursors for a group of biologically active molecules termed the eicosanoids, which include the prostaglandins (PG), leukotrienes, thromboxanes and prostacyclins. These hormone like molecules are involved in many metabolic pathways in the body (Palmquist, 2009).

2.2.1.1 Eicosanoids

The eicosanoids are derived from 20 carbon PUFA's, and act as local hormones (generated in situ and elicit effects within the immediate vicinity due to rapid metabolism). They are involved in the maintenance, growth, reproduction and health of animals, and are essential to life (Lands, 1992). Collectively, the eicosanoids are involved in modulating the release of hypothalamic and pituitary hormones including growth hormone, prolactin, antidiuretic hormone, leutenizing hormone and follicle stimulating hormone (Lands, 1992), and are associated with thrombosis events such as heart attacks and strokes (Holub, 2002), immune and inflammatory reactions including arthritis (Caughey et al., 1996), lupus and asthma, dysmenorrhea (Lands, 1992) and certain cancers such as colorectal (Baro et al., 1998), prostate (Newcomer et al., 2001) and breast cancer (Klein et al., 2000), all of which are associated with increased n-6 derived eicosanoid production.

More specifically, prostaglandins are involved in platelet aggregation, blood vessel constriction/dilation, inflammatory and immune responses and play major roles in reproduction (Gurr et al., 2002). Prostaglandin $F_{2\alpha}$ is a leuteolytic hormone and is thus involved in the maintenance of pregnancy and the initiation of parturition (Allen and Harris, 2001), and PGE_2 inhibits ovulation (Wathes et al., 2007). Leukotrienes and lipoxins are involved in inflammatory processes, chemotaxis and vascular permeability, but may also play a role in the onset of labor (Allen and Harris, 2001). Smooth muscle contractions, platelet aggregation and cell adhesion to blood vessel walls are in part regulated by several of the thromboxanes (Gurr et al., 2002). There are over 20 known biologically active eicosanoids, including those formed from the n-3 and n-6 pathways (Lands, 1992).

As discussed above, the eicosanoids are formed using 20 carbon ArA or EPA as a precursor. Excess PUFA's are stored in cell membranes in the body in an esterified form, and thus the initial step of eicosanoid formation is to liberate the PUFA substrates from the phospholipid membranes via the action of phospholipase A2 (Lands, 1991). Once ArA or EPA are liberated from the phospholipid membrane they can enter several different pathways for eicosanoid synthesis. Formation of leukotrienes or lipoxins requires the action of lipoxygenase enzymes, and production of prostacyclin, prostaglandins and thromboxanes requires a two step process initiated by cyclooxygenase to create a ring structure (Voet et al., 2008). Following the formation of cyclic endoperoxides, prostacyclin synthetase forms the prostacyclin family of eicosanoids, while reductases or isomerases form the prostaglandins, and thromboxane synthetase leads to the formation of the thromboxane family (Gurr et al., 2002).

In addition to the overall synthesis pathways, differences exist in the final eicosanoid produced depending on the precursor FA used (ArA vs. EPA). In general, the molecules formed from the n-6 precursor (2-series PG's, 4-series leukotrienes and 2-series thromboxanes) have greater biological activity relative to those formed from the n-3 precursor (3-series PG's, 5-series leukotrienes and 3-series thromboxanes) (Gurr et al., 2002; Lands, 1992; Wathes et al., 2007). For example, $PGF_{2\alpha}$ usually has 10-fold greater potency compared with $PGF_{3\alpha}$ (Wathes et al., 2007). Enzymes involved in the conversion of the 18 carbon PUFA's into their longer chain counterparts have greater affinity for the

n-3 FA's relative to the n-6 FA's (Palmquist, 2009). Thus, for example, when EPA is present in sufficient amounts, it can depress the metabolism of n-6 PUFA's and hence the synthesis of the 2-series PG's.

2.2.1.2 Reproduction

Reproduction is a highly complex, integrated system of processes involving many different hormones and molecules (Senger, 2003). Some of the major reproductive hormones include those of the prostaglandin family, formed from their PUFA precursors. As an example, one role of $PGF_{2\alpha}$ is to trigger luteolysis during estrus and pre-parturition (Senger, 2003). Luteolysis must be prevented in order for an animal to remain pregnant, and thus throughout pregnancy, the production of $PGF_{2\alpha}$ must be removed, reduced or re-routed (Senger, 2003). There are many signals, hormones and other molecules which are involved in this complicated process; however, it may be possible to aid in the maintenance of pregnancy by increasing the level of $PGF_{3\alpha}$ produced at the expense of $PGF_{2\alpha}$ (Ambrose et al., 2006).

Over the last 10-15 years, several studies have examined the effects of PUFA's on reproduction. There are several review papers discussing the effects of n-3 FA's on the reproductive processes of animals and humans (Abayasekara and Wathes, 1999; Allen and Harris, 2001; Wathes et al., 2007). In cattle, alteration of dietary PUFA content can alter the number and size of pre-ovulatory follicles, ovulation rate, conception rate, progesterone production by the corpus luteum and $PGF_{2\alpha}$ production which in turn affects luteolysis and the length of gestation (Petit and Benchaar, 2007; Petit et al., 2002; Petit and Twagiramungu, 2006). Reductions in abortion and pregnancy losses have also been reported (Ambrose et al., 2006).

Inclusion of dietary n-3 FA's into sow diets increased the PUFA content in sow and piglet plasma and in milk (Fritsche et al., 1993; Rooke et al., 2001a), which, in turn, has been attributed to improving pre-weaning survival. Additionally, Webel et al. (2003) showed an increase in the number of piglets born alive when sows were fed fish oil.

In summary, PUFA's have demonstrated potential for improving the reproductive performance of sows and other animals. Results, however; have been variable, and thus a better understanding of how dietary PUFA's affect animal reproductive processes is needed.

2.2.1.3 Immunity

As well as influencing the eicosanoids, intake of n-3 FA's also alters the circulating profile of cytokine molecules (Simopoulos, 2002). Cytokines are immune cell proteins which are secreted in response to stimuli such as pathogen and stress, and assist in regulating the development of an immune or inflammatory response (Tizard, 2009). They can act on themselves (autocrine) or locally (paracrine) to elicit responses from other immune cells, and are produced by monocytes and macrophages (Tizard, 2009). Omega-3 FA's can act upon intracellular signaling pathways, transcription factor activity and/or gene expression to modulate cytokine function (Simopoulos, 2002). Essentially, interactions between immune and inflammatory cells are mediated by cytokines. Tumor necrosis factor (TNF-α), interleukin (IL)-1, IL-6 and IL-8 are some of the primary pro-inflammatory cytokines found in the body (Webel et al., 1997). Production of appropriate amounts of cytokines must occur in response to immunologic stimuli for animals to remain healthy and fight off infections; however, over or inappropriate production of these cells can be detrimental. It has been well documented that production of the pro-inflammatory cytokines will increase muscle degradation, reduce protein synthesis and divert nutrients to the synthesis of other immune molecules (Zhan et al., 2009). The n-3 FA's are considered to be anti-inflammatory, and several studies have shown that dietary inclusion of n-3's can lead to altered production of some of the pro-inflammatory cytokines (Cotogni et al., 2011; Dunstan et al., 2004; Wallace et al., 2001).

There is also some evidence that PUFA's can have effects on the production of immunoglobulins (Ig) in mammals; however, the mechanism is not clearly understood. A study by Dunstan et al. (2004) found that women fed fish oil had increased IgA concentrations in their milk, while Jackson et al. (1995) showed that corn fed sows (high n-6 FA's) had depressed IgG concentrations in their milk. A recent article by Mateo et al.

(2009) presented results of increased IgG concentrations in the colostrum and milk of sows fed a fish product. Clearly, dietary n-3 and n-6 FA's are involved in the immune and inflammatory processes, and thus may be an important dietary target for improvement of animal performances.

2.2.1.4 Energy Source

As with any FA, n-6 and n-3 FA's can be oxidized and used as a source of energy. Circulating free FA's and those hydrolyzed from triacylglycerides provide energy via β-oxidation (Voet et al., 2008). With each step of the pathway, a 2 carbon acetyl-CoA is generated which then enters into the tricarboxylic acid cycle. During this cycle, each 2 carbon acetyl-CoA molecule generates reduced pyridine nucleotides which then pass into the mitochondrial electron transport chain for the generation of ATP (Voet et al., 2008).

Additionally, there is some evidence that dietary n-3 FA's affect glucose absorption (Aas et al., 2006; Gabler et al., 2009; Gabler et al., 2007) and improve insulin-mediated glucose metabolism in animals (Borkman et al., 1993; Gingras et al., 2007), and energy utilization. Clarke (2001) has also discussed the role of n-3 FA's as 'fuel partitioners', since they can act on genes which promote FA oxidation and down regulate those involved in lipogenesis.

2.2.2 Omega-3 & Omega-6 Fatty Acids

The n-3 and n-6 FA's are considered nutritionally essential. Mammals are incapable of synthesising the 18 carbon omega FA's, and they must be consumed in the diet (Gurr et al., 2002). There is some capacity within the body for the synthesis of the longer chain derivatives; however the efficiency of this process varies among species (Martinez-Ramirez et al., 2008), and is dependent on dietary n-6 and n-3 intake (Harnack et al., 2009).

2.2.2.1 Dietary Sources

The most common dietary sources of n-3 PUFA's include marine and land based sources. Common plant based sources of n-3 FA's used in animal nutrition include corn, soybean, canola and flaxseed products (seed, meal and/or oil) (Palmquist, 2009). Corn and soybean are also high in n-6 FA's, as LA comprises 55- 57% of the total FA's (NRC, 1998). Canola contains only 22% of its total fat as LA and 11% as ALA (NRC, 1998). Flaxseed is the richest land based source of n-3 FA's, with over 55% of its total fat consisting of ALA (Doppenberg and van der Aar, 2009). The majority of plant based omega FA's are 18 carbons, and EPA, DHA and ArA are not typically present.

Arachidonic acid may be consumed in animal sources of fat such as lard or tallow, or is synthesized from its 18-carbon precursor within the body. The long chain n-3 FA's EPA and DHA can be obtained in the diet through consumption of marine products (Palmquist, 2009). Fatty fish such as herring, anchovy or menhaden contain over 20% of their FA's as n-3's, the majority of which are of carbon chain length greater than 20 (NRC, 1998), and contain very little n-6 FA's (< 2%). Algae are also a good source of EPA and DHA (Palmquist, 2009).

2.2.2.2 *De Novo* Synthesis of Long Chain PUFA's and the Importance of the n-6:n-3 Ratio

The n-3 FA's typically fall into two groups, the less biologically active ALA, as well as DHA and EPA which are believed to be the n-3's responsible for many of the health and reproductive benefits described above (Palmquist, 2009). The 18 carbon omega FA's must be consumed in the diet as they cannot be synthesized. However, DHA and EPA can be from one of two origins, either from direct consumption (ex. fish oil), or through biosynthetic pathways utilizing dietary ALA as the precursor. Similarly, ArA can be consumed directly or synthesized within the body using LA as its precursor (Palmquist, 2009).

De novo synthesis of DHA and ArA utilizes a series of steps involving desaturation and elongation enzymes (Palmquist, 2009). The formation of the long chain n-6 and n-3 FA's from their shorter precursors is competitive as they utilize the same set of enzymes. The enzymes have a greater affinity for the n-3 than n-6 FA's (Palmquist, 2009). Typical western diets for humans and livestock however, contain 10 to 25 times more n-6 (Simopoulos, 1991), essentially diluting the n-3's and leading to the formation of greater quantities of ArA than EPA. Historically, it is believed that humans and animals consumed diets with an n-6:n-3 ratio of 2:1 or 1:1 (Allen and Harris, 2001), thus leading to the belief that the current dietary n-6:n-3 ratios must be reduced in order to improve overall health and animal performance.

Estimates of the efficiency of conversion of ALA into its longer chain counterparts in humans are variable (Brenna et al., 2009; Burdge and Calder, 2005; Welch et al., 2008); however, it is apparently inefficient. In the swine literature, there are few estimates of the efficiency of this conversion process. However, Martinez-Ramirez et al. (2008) have shown it to be more efficient than what is reported for humans or rodents.

The conversion of ALA into EPA and DHA is more dependent on the ratio of the FA's in the diet than the absolute amount (Clarke, 2001; Harnack et al., 2009). In theory, reducing the dietary ratio of n-6:n-3 FA's would reduce competition between the conversion pathways for the desaturase and elongase enzymes, thus allowing for increased conversion of ALA into EPA. However, simple addition of a small amount of n-3 into a diet high in n-6 may not lower the ratio sufficiently to observe improvements in conversion efficiency, and thus an optimal ratio should be determined.

2.3 PUFA's in Swine Rations

Recently, there has been a growing interest to adopt the use of dietary long chain PUFA's by the pork industry. A large part of this is due to increased awareness of the putative health benefits of n-3's, as well as consumer demand for value-added pork products.

In monogastric animals such as pigs, dietary FA's are absorbed from the gastrointestinal tract relatively unchanged, and thus the carcass FA profile reflects the FA profile of the diet (Fickova et al., 1998). Altering the FA carcass composition of monogastric animals through dietary inclusion of n-3 FA's may therefore lead to a greater consumption of n-3 FA's by the consumer. In addition, since these FA's are precursors for many metabolic hormones and compounds in the body, they can also alter the metabolic processes within the animal, potentially improving health and performance.

2.3.1 The Sow

Many studies have shown that inclusion of dietary n-3 FA's into sow diets can increase PUFA content in colostrum, milk and plasma as well as in the tissues of offspring (Arbuckle and Innis, 1993; Fritsche et al., 1993; Lauridsen and Danielsen, 2004). Some studies have shown improvements in piglet growth and reductions in pre-weaning mortality (Rooke et al., 2001a,b); inconsistencies however, among studies, have been reported and may be due to the high variability in control diet composition, length of time of feeding, or farm management practices. Other studies have shown increases in the number of piglets born alive when sows were fed diets containing a fish based n-3 source during the previous reproductive cycle (Smits et al., 2011; Webel et al., 2004). In many of these studies a corn based control diet was fed, which is high in n-6 FA's and thus the high n-6:n-3 ratio may have prevented observation of the benefits of feeding n-3 FA's to sows during gestation and lactation.

Lactational homeorhesis refers to the physiological drive to produce milk at the expense of other body functions. It is well researched in the modern dairy cow, but also is characteristic of the metabolic state of lactating sows undergoing a period of negative energy balance immediately following parturition (Pettigrew et al., 1993). During this state of negative energy balance, the animal relies on adipose tissue mobilization to support milk production (Pettigrew et al., 1993), and thus ensure adequate nutrients are supplied to the offspring. In addition to the increased metabolic demand placed on the sow at the onset of lactation, hypophagia (reduced feed intake), immediately post-

farrowing, contributes to the inability of the animal to meet nutrient demands for maximal milk production.

Hormonal regulation of appetite and milk production may affect the degree of negative energy balance encountered by modern sows. In the sow, high feed consumption during gestation results in reduced feed intake during the subsequent lactation (Weldon et al., 1994a; Weldon et al., 1994b). Several explanations for this have been suggested including roles for circulating hormones and adipose tissue adaptation. When sows were fed either a standard restricted diet, or *ad libitum* from d 60 of gestation until farrowing, the sows fed *ad libitum* during gestation gained more weight during gestation but lost more weight during lactation (Weldon et al., 1994a). During lactation, insulin secretion was increased and plasma NEFA concentration was reduced in the restricted fed sows. The authors (Weldon et al., 1994a) concluded that higher insulin concentration in sows fed the restricted diet during gestation may have stimulated appetite by reducing lipid mobilization and increasing peripheral glucose use.

Other hormones which may regulate feed intake around the time of parturition include ghrelin and leptin, and while evidence doesn't support a role for ghrelin (Govoni et al., 2007) there is evidence to suggest that leptin is important. Leptin, a hormone secreted by adipose tissue, is an important regulator of appetite, energy metabolism, and body composition (Hossner, 1998). When adipose tissue reserves are high, leptin activates satiety centers in the hypothalamus, and food intake is reduced (Hossner, 1998). Studies have shown a positive correlation between leptin levels and backfat thickness in growing pigs (Robert et al., 1998) and sows (Estienne et al., 2000). Negative energy balance in sows resulted in a rapid decrease in leptin concentrations in plasma and adipose tissue (Estienne et al., 2000). Insulin, glucose and IGF-I, all reduced in the fasted state, are potent regulators of leptin and a positive correlation was observed between plasma insulin, leptin and luteinizing hormone concentrations in lactating sows fed *ad libitum* compared to feed restricted sows (Barb et al., 2005).

While there is a large amount of information available on whole body responses to lactation in the dairy cow, there is very little available on sow's responses. McNamara (1991) has clearly demonstrated that following parturition in the dairy cow, a dramatic increase in catabolism and decrease in anabolism occurs. Although this is in part a whole

body response to the period of negative energy balance, adipose tissue responsiveness is also altered during this critical time (McNamara and Hillers, 1986), with reduced lipogenesis and FA esterification, and increased free FA release in adipose tissue samples collected two weeks post-partum relative to those collected four weeks post-partum.

As discussed above, in monogastric animals such as pigs, the FA profile of body fat reflects the FA composition of the diets (Eastwood et al., 2009; Fickova et al., 1998; Romans et al., 1995a; Romans et al., 1995b). Fickova et al. (1998) reviewed the role of using n-3 FA's to replace n-6 FA's in reducing plasma triglycerides. However, there is little data available on the effects of dietary n-3 FA inclusion on lipogenesis or lipolysis. Lipolysis was decreased when rats were fed PUFA enriched diets for eight weeks (Gavia et al., 2001). Rats fed n-3 enriched diets for just one week exhibited increased lipolytic response and decreased insulin-stimulated glucose transport and lipogenesis (Fickova et al., 1998). In other studies, dietary FA composition had no effect on body fat metabolism. Awad et al. (1990) was unable to show any effect of dietary FA composition on lipid metabolism in the adipose tissue of mature rats which is similar to the early work of Allee et al. (1972), who was unable to demonstrate an effect of dietary FA composition on lipogenesis in pigs. In another study, 10 % tallow added to the diet of lactating sows did not alter the rates of lipolysis measured using an exogenous epinephrine challenge (Tilton et al., 1999). Papadopoulos et al. (2009b) looked at the effects of supplementing a lactation feed with 2 % oil (high n-6:n-3 ratio) administered eight days pre-parturition. They found that the fat source was associated with high serum leptin concentrations pre- and post-partum, insulin resistance on the first day of lactation, and decreased feed intake on the first and second days following parturition. Moreover, across all treatment groups, there was a significant negative correlation between leptin and litter weight and litter growth.

Across species, the effects of n-3 FA's on lipogenesis and lipolysis are not well understood, with very little information available from sows. Moreover, available results are variable and thus the role of body FA composition on adipose tissue metabolism and whole body responses post-farrowing is unclear.

2.3.2 The Piglet

The use of PUFA's for piglets can be divided into two stages, lactation and post-weaning. During lactation, piglets obtain PUFA's through the milk of their dam. As discussed above, in monogastric animals, the FA profile of the carcass and milk, mimics dietary intake. Thus, feeding sows a diet enriched with n-3 FA's will lead to the production of n-3 enriched milk, which is then consumed by the offspring (Rooke et al., 1998, 2000). Post-weaning, piglets can obtain n-3 FA's from supplemented nursery diets.

There are several reasons why producers may feed n-3 FA's to piglets. As discussed above, enriching sow diets with n-3 FA's has led to increased pre-weaning survival, greater piglet growth during lactation, greater birth and weaning weights and higher piglet survivability at birth (Leonard et al., 2010a; Rooke et al., 1998; Rooke et al., 2000; Smits et al., 2011). Additionally, n-3 FA's may alter inflammatory and immune responses (Gaines et al., 2003; Liu et al., 2003), and thus producers may include n-3 FA's into piglet diets with the aim of reducing the use of prophylactic antibiotic treatment in starter feeds. With such a wide array of functions in the body, the use of PUFA's in piglet diets has great potential to improve animal performance and health.

Stresses at weaning contribute to the 'post-weaning growth lag', which is characterized by piglets having reduced feed intake for 24 to 48 hours post weaning as they transition to solid feed, reduced growth, and increased susceptibility to pathogens (Patience et al., 1995). The major stressors, removal from the sow to a different facility, mixing with unfamiliar animals, a new, unfamiliar feed and exposure to different bacteria and pathogens, all lead to activation of the innate immune and inflammatory responses. Discussed in detail above, inclusion of n-3 FA's into animal diets can lead to alterations in these immune responses and thus may be a target for helping to alleviate the post-weaning growth lag observed in many barns. The n-3's are anti-inflammatory, and thus may reduce the excess inflammatory response often observed at this time period.

2.4 Lipopolysaccharide Bioassay

In order to study the inflammatory and innate immune reactions of animals, a safe, easy to use model is required that can mimic the normal responses of animals exposed to stress or pathogenic stimuli. One such model utilizes *E. coli* based lipopolysaccharide (LPS). Lipopolysaccharide is a component of the outer wall of Gram negative bacteria, and is detected in the body by pattern recognition receptors and toll like receptor (TLR) 4 (Tizard, 2009). Once detected, it triggers a cascade of events and elicits a generalized inflammatory reaction, characterized by rapid increases in circulating pro-inflammatory cytokines such as TNFα, IL-1, IL-6 and IL-8 (Webel et al., 1997). Additionally, LPS causes a transient rise in body temperature, which returns to normal post-challenge (Rakhshandeh and de Lange, 2012). Williams et al. (2009) describes LPS as also triggering the hypothalamic-pituitary-adrenal (HPA) axis, causing the increased production of the stress hormone, cortisol.

Lipopolysaccharide can be used in several ways. The location of injection may vary (intra-peritoneal, intra-muscular, subcutaneous, etc), as can the amount and frequency of injection. Injections of large amounts will cause high mortality; however, when injected in small doses, LPS can be used as a model for moderate or chronic (when injected over multiple days) inflammatory and innate immune responses (Rakhshandeh and de Lange, 2012). Despite generating an immune response which mimics that of an immunologically or stress challenged animal, LPS is only one component of the bacterium and does not contain the ability to replicate, making it safe for use in healthy herds (Rakhshandeh and de Lange, 2012).

2.5 Cytokine Assays

Cytokines can be detected and quantified using several different techniques. The three main assays used are the enzyme-linked immunosorbent assay (ELISA), SearchLight® technology by Aushon Biosystems (Billerica, MA) and fluorescent microsphere immunoassay (FMIA). The most common method used is the ELISA.

The ELISA test works when antibodies to a specific protein (in this case, specific cytokines) bind to that protein and the bound antibodies are detected by an enzyme-labelled antiglobulin. When the enzyme substrate is added into the mixture, a colour change occurs which is proportional to the amount of bound antibody (Tizzard, 2009). The colour change can then be estimated either visually or using an ELISA reader, which is a modified spectrophotometer. This method is relatively easy and thus the most common method for cytokine analysis; however, it may not be the most consistent and accurate method available.

The SearchLight® technology developed by Aushon Biosystems is a protein array technology which used a sandwich-ELISA and chemiluminescent or fluorescent detection systems (www.aushon.com). The system works on a micro-scale and can measure up to 16 different cytokines simultaneously on a single sample. The system is fast, accurate, sensitive and reliable, and can be customized.

The final main assay used for cytokine analysis is the FMIA. As with the SearchLight® technology, this is a multi-plex system which can analyze multiple proteins simultaneously from a single sample; however, the methods are slightly different. In this case, fluorescently labelled polystyrene beads are coated with an antigen and mixed with the sample, allowing for binding to the proteins of interest. The technology uses flow cytometry to categorize bead types and quantify the level of bound antibodies (Lawson et al., 2010). This method is rapid, accurate and sensitive; however, it is still within the development stages for certain sample types.

2.6 Summary

Overall, it is important for pork producers to maximize the reproductive performance of their sows. Improving animal performance over extended periods of time will not only maximize pork output, but will also increase herd longevity, maximizing income on a per sow basis. Maximizing piglet growth, survivability and overall health has major economic benefits for the producer.

Recently, we have seen an increase in the use of novel or 'functional' feeds with the aim of improving animal health, performance and reducing cost of production. The use of PUFA's in animal rations has been gaining momentum over the last 10 to 15 years, and more rapidly in the last 5 years. The use of n-3 FA's is of growing interest due to putative health benefits for animals and consumers of the pork products. Based on the literature, providing n-3 FA's to pigs can improve sow reproduction, piglet performance and animal health; however, with reported inconsistencies. The later may potentially be due to the fact that many studies use diets high in n-6 FA's which may 'wash out' potential benefits of including n-3's. The ratio of the two FA's may be more important than the absolute amount provided in the diet, as the n-6:n-3 ratio decreases, animals may have an increased ability to convert the 18 carbon ALA into its longer chain, more biologically active counterparts (EPA and DHA). It is also possible to speculate that plant based sources of n-3 FA's (such as those found in flaxseed), may become a reliable source of n-3 FA's for animal feeds, for a fraction of the price of fish based sources.

In conclusion, n-3 FA's have potential for use in swine diets. The current literature however, is variable and no recommendations can be made on inclusion levels, source, or the correct ratio relative to n-6 FA's. The experiments detailed in the forthcoming chapters were designed to answer some of these questions, and provide additional insight into some of the mechanisms behind the dietary benefits of n-3 FA's to sows and piglets.

3 HYPOTHESIS & OBJECTIVES

It is hypothesized that reducing the n-6 to n-3 FA ratio in sow diets from 9:1 to 1:1 improves sow reproductive performance and lessen the inflammatory responses of their offspring post-weaning. For the purpose of these experiments, 'improved reproductive performance' is defined as increased piglets born alive, increased piglets weaned, as well as heavier body weights at farrowing and throughout lactation.

To test the overall hypothesis, the project was divided into three experiments, each with their own specific hypothesis and objectives.

Experiment 1: *Effects of altering the n-6 to n-3 FA ratio in sow diets on reproductive performance, serum and colostrum fatty acid profiles and passive immunity*

This experiment was designed to test the hypothesis that reducing the n-6 to n-3 FA ratio in sow diets from 9:1 to 1:1 increases circulating concentrations of n-3 FA's in sows and their offspring, and improves the passive immune status, characterized by increased circulating immunoglobulins of piglets.

The objectives were to determine:

- If the FA profile in sow and piglet blood, colostrum and milk are altered in sows fed diets with varied n-6 to n-3 FA ratios
- If the source of the n-3 FA's (fish or flax) differentially impacts circulating concentrations of long chain n-3's (EPA and/or DHA)
- If IgA and IgG concentrations in colostrum and piglet serum are affected by the n-6 to n-3 FA ratio of gestating sows
- If farrowing and weaning rates, piglet survival and growth performance are altered when the sows consumed varied n-6 to n-3 FA ratios during gestation and lactation

Experiment 2: *Effects of altering the n-6 to n-3 fatty acid ratio in sow diets on body fat mobilization during lactation*

It is hypothesized that prolific sows (≥ 11 piglets farrowed, ≥ 10 piglets nursing) consuming diets with n-6 to n-3 FA ratios reduced from 9:1 to 1:1 throughout gestation and lactation have increased body fat mobilization and increased milk nutrient yield.

The objectives were to determine if changing n-3 FA intake relative to n-6 FA intake affects:

- Loss of backfat during lactation
- Milk production
- Piglet growth performance during lactation

Experiment 3: *Effects of altering the n-6 to n-3 FA ratio in sow diets on the inflammatory responses of their offspring post weaning when challenged with an E. coli lipopolysaccharide*

It is hypothesized that piglets raised by sows consuming diets with reduced n-6 to n-3 ratios during gestation and lactation have reduced acute inflammatory responses post-weaning.

The objectives were to determine if the n-6:n-3 ratio of the sow diet affects the febrile and pro-inflammatory cytokine responses of their piglets one week post weaning when challenged with an *E. coli* based lipopolysaccharide.

4 EFFECTS OF ALTERING THE OMEGA-6 TO OMEGA-3 FATTY ACID RATIO IN SOW DIETS ON REPRODUCTIVE PERFORMANCE, SERUM AND COLOSTRUM FATTY ACID PROFILES, AND THE CONVERSION OF α-LINOLENIC ACID INTO EICOSAPENTAENOIC ACID

4.1 Abstract

An experiment was designed to test the hypothesis that reducing the sow dietary omega-6 (n-6) to omega-3 (n-3) fatty acid (FA) ratio improves reproductive performance, characterized by improved weaning rates, piglet survival and growth performance. Additionally, we aimed to determine if the FA profile of sow and piglet blood, colostrum and milk are altered in sows fed diets with varied n-6:n-3 ratios, and if the dietary FA ratio and n-3 source impacts circulating concentrations of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid.

Sows (n=150) were randomly assigned to one of five diets on d 80 of gestation. Period 1 (P1) is the period from d 80 of gestation to weaning, and period 2 (P2) refers to d 1 post-weaning to the subsequent weaning. Wheat/barley diets (5% crude fat) with varied amounts and ratios of polyunsaturated fatty acids (PUFA) were fed to gestating and lactating sows. The treatment diets consisted of a control diet (tallow based, similar to a standard production diet), 3 diets with plant oil based n-6:n-3 ratios (9:1P, 5:1P, and 1:1P) and a 5:1 diet based on fish oil (5:1F). The control diet had a ratio of 8:1 but contained approximately 50% less PUFA than the other diets. Performance data was collected throughout each period, and data was analyzed using a completely randomized design. Fatty acid composition of sow and piglet serum, colostrum and milk was measured. IgA and IgG were determined in colostrum and piglet serum.

The number of piglets born per litter (total born and live born) within each period was unaffected by diet ($P > 0.05$). In P1, birth weights were unaffected by diet ($P > 0.05$). Average piglet weaning weight ($P = 0.02$) and ADG ($P = 0.01$) were greatest for piglets born to sows consuming the 9:1P and 5:1P diets, intermediate for piglets born to control fed sows, and lowest for piglets born to 5:1F diet sows. During P2, 5:1F sows consumed 10% less feed ($P = 0.04$), had reduced piglet birth weights ($P = 0.05$), and average piglet weaning weight was reduced by 0.8 kg ($P = 0.04$) relative to sows on each of the other diets. Stillbirths where higher in all diet groups relative to the control group ($P = 0.02$).

Colostrum and piglet serum IgA and IgG concentrations were unaffected by diet ($P > 0.05$). Colostrum FA profiles reflected the pattern in sow diets. Colostrum from sows fed the 5:1F diet had lower total n-3 FA's compared with the colostrum produced from sows fed the plant based diets, although the n-3 profile was different ($P < 0.01$). Total serum n-3 FA's were greatest in sows consuming 1:1P and 5:1F diets, and in their offspring ($P < 0.01$). In sows, ALA was highest in the 1:1P group and EPA and DHA were highest in the 5:1F group. In pre-suckle piglet serum, ALA and DHA did not differ among treatment groups ($P > 0.05$). Relative to piglets of sows consuming the control diet, EPA was 2.5 times greater in the 1:1P sows and 4 times greater in the 5:1F fed sows ($P < 0.01$) prior to suckling. In post-suckle samples, ALA was highest in piglets from the 1:1P fed sows ($P < 0.01$), and EPA and DHA were highest in piglets from the 5:1F fed sows ($P < 0.01$).

Reproductive performance of sows consuming the plant based diets (9:1P, 5:1P and 1:1P) was similar to that of sows consuming the control diet; however, sows consuming the 5:1F diet had smaller piglets at birth and weaning, and consumed less feed relative to all other sows. Performance of sows consuming the 5:1P diet did not differ from those consuming the control diet, whereas sows consuming the 9:1P and 1:1P diets were intermediate between the control, 9:1P sows and 5:1F sows for some parameters. Increasing the intake of plant based n-3 FA's in a 1:1 (n-6:n-3) ratio increased circulating levels of EPA in addition to ALA in both sows and piglets relative to the other plant based diets. Overall, reproductive performance was unaffected.

Key Words: omega-3, omega-6, colostrum, milk, piglet, sow

4.2 Introduction

In the swine industry, two of the most critical stages in the production cycle are the breeding and the farrowing to weaning periods. Modern sows are very prolific, and due to steady increases in litter size, the productivity of the pork industry is continually improving (PigChamp, 2011). This increase in litter size, however, has been accompanied by negative consequences on piglet survival and/or performance (Boulot et al., 2008), as well as on sow longevity (Peet, 2008), thus producers cannot take full advantage of the increases in litter sizes.

There has been increasing interest in the use of dietary polyunsaturated fatty acids (PUFA), specifically the omega-3 (n-3) fatty acids (FA) α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to improve sow performance. The PUFA's are precursors for different hormones and molecules including the eicosanoids, and thus they influence many components of the biological system (Lands, 1992). The specific n-3 FA provided in the diet may also be important, as prior to formation of the eicosanoids, ALA (found in plant sources such as flaxseed) must first be converted into EPA (found in marine sources), which in turn suggests that the longer chain n-3's are more biologically active. Both the n-3 and n-6 FA's are involved in reproduction (Allen and Harris, 2001; Wathes et al., 2007) and health (Calder, 2001; Palmquist, 2009). There is a direct competition between the n-3 and n-6 FA's for the enzymes involved in the synthesis of the biologically active longer chain derivatives, and thus the ratio may be important in maximizing the benefits of including 18 carbon n-3 FA's into the diet (Palmquist, 2009). Importantly, studies looking at the conversion of ALA into EPA and DHA have shown that conversion is dependent on the n-6:n-3 FA ratio in the diet, not on their absolute intake (Harnack et al., 2009).

This experiment is designed to test the hypotheses that reducing the n-6:n-3 FA ratio in sow diets improves reproductive performances (characterized by increased piglets born alive, increased numbers weaned, as well as heavier body weights at birth and throughout lactation). Specifically, it is hypothesized that reducing the n-6:n-3 ratio in sow diets increases circulating concentrations of n-3 FA's in sows and their offspring, and improves the passive immune status of piglets.

4.3 Materials and Methods

4.3.1 General

This experiment was approved by the University of Saskatchewan's Animal Research Ethics Board, (UCACS #'s 19970020 and 20090040) and adhered to the Canadian Council on Animal Care guidelines for humane animal use (CCAC, 1993). The experiment was conducted at the Prairie Swine Centre Inc. (Saskatoon, Saskatchewan, Canada). All animals used throughout this trial were housed in temperature-controlled rooms according to the thermoneutral zone for the specific age and stage of reproduction (Zhang, 1994). Lighting was maintained on a 12 h light:dark cycle (0700 – 1900). All pigs were a commercial genetic line (Camborough Plus females x C3378 sires, PIC Canada Ltd., Winnipeg, Manitoba, Canada). The flaxseed meal (FSM) and flaxseed oil (FSO) were obtained from a commercial company (Vandeputte S.A., Mouscron, Belgium). The composition of this FSM product was previously reported by Eastwood et al. (2009). Corn oil was obtained from Pestell Minerals & Ingredients (New Hamburg, Ontario, Canada), and 0.25 g/kg of ethoxyquin was added to the corn oil upon arrival as an antioxidant. Herring oil was provided by the commercial feed mill used to make diets (Federated Coop, Saskatoon, SK). Unless otherwise stated, all laboratory analyses were conducted at the Department of Animal and Poultry Science, University of Saskatchewan.

4.3.2 Animals and Housing

A total of 150 sows (240 ± 33 kg, initial parity 0 to 4) were utilized (n = 30/treatment) in a completely randomized block design. Sows were assigned to diets based upon their expected farrowing dates, and treatment groups were blocked across parities. A total of 8 to 12 sows were available each week for use on the trial, which included all healthy sows within the required parity range. Sows were managed according to standard production practices during breeding, gestation and lactation, and all piglets

were cared for under normal operating procedures. Any cross-fostering of piglets occurred within the first 24 hours of farrowing, and piglets could not always remain within treatment group when cross-fostered depending upon available sows. Lactation averaged 26 ± 2 days. At trial onset, sows were ear-tagged with one of 5 colours, each corresponding to that sows respective diet for easy identification. If a sow had to be removed due to adverse health issues, a replacement sow was used who began at the start of the trial (P1) to ensure she was on her respective test diet for the same length of time prior to data collection, and the original sow was removed from the data set.

During gestation all animals were group housed in a free access stall system (INN-O-STALL Free Access Stall, Egebjerg International, Denmark). Each cohort of animals contained 2 breeding groups and had farrowing due dates spanning 2 weeks. The gestation facility contained 6 group pens, each with 32 individual, walk in/lock in stalls (2 rows of 16 stalls). There were 2 types of group pens in the facility. Four were 'T' pens and the remaining 2 were 'I' pens. The 'I' pens had fully slatted concrete floors while the 'T' pens contained a solid floor loafing area in addition to the slatted 'I' shaped area. Sows were able to come and go freely from their stalls while housed in this system. Each stall measured 66 cm wide by 210 cm long (front 150 cm solid floor, back 60 cm slatted) by 103 cm high. The stalls were equipped with an individual feeder as well as a nipple drinker. Diagrams are shown in Appendix A.

On d 110 of gestation, sows were moved from the group housing gestation facility to a farrowing room equipped with 16 individual farrowing crates. Each crate measured 183 cm wide by 244 cm long and had an adjustable sow space as well as a piglet creep area (INN-O-CRATE Farrowing Crate, Egebjerg International, Denmark). The flooring was fully slatted metal and contained rubber mats in the piglet areas. Crate siding was polyvinyl chloride (PVC) as was the creep area hood. Heat lamps were provided in the creep area for the piglets. The adjustable sow area was fitted with metal bar siding and crash bars, and could be adjusted in length (190 to 201 cm) and width (57 to 85 cm at back) to accommodate sows of various sizes. The piglet area contained an easy access hood, a heat lamp and measured 90 cm by 90 cm. Individual bowl feeders and nipple drinkers were located at the front of each sow space and creep feed was provided for the final week of lactation. Diagrams of the farrowing facility are shown in Appendix A.

After weaning, sows were moved into the breeding room equipped with individual stalls (CADILLAC AI Stall, Egebjerg International, Denmark). There were three different sizes of stalls designed to accommodate sows of varying sizes. Each stall was 208 cm long and either 61 cm, 66 cm or 76 cm in width. The stalls contained partially slatted concrete floors and an individual bowl feeder and nipple drinker (see Appendix A). Post-weaning the sows were exposed to a boar daily (head to head contact) until standing heat was detected at which point they were bred using artificial insemination (PG 600® was not utilized during the trial). Pregnancy was confirmed via ultrasound at approximately day 20 post-breeding and sows were moved into the gestation group housing facility at 4 to 5 weeks pregnant.

4.3.3 Treatments and Feeding

The 5 dietary treatments were each divided into a gestation and a lactation ration (10 diets). Diets were formulated to be balanced for net energy and digestible essential amino acids according to NRC recommendations for gestating and lactating sows (NRC, 1998). The diets were formulated based on the digestible oil content, to contain equal amounts of crude fat (5%). Each diet was wheat and barley based, and contained additional vitamins, minerals and amino acids to meet the nutrient requirements of the sows. Ethoxyquin was added (0.025% inclusion) to all diets to reduce the risk of FA oxidation. All diets were pelleted.

Fatty acids were supplemented at differing levels to adjust the ratio of n-3 to n-6 FA’s in the diets. The FA’s of interest were the n-6 FA linoleic acid (LA), the main source of which was corn oil, and the n-3 FA ALA, the sources of which were flax oil and flaxseed meal. Fish oil (herring) was used as a source of long chain n-3 FA's (EPA and DHA). The treatment groups consisted of a control diet (tallow based, similar to a typical production diet), 3 diets with plant oil based n-6:n-3 ratios (9:1P, 5:1P, and 1:1P) as well as a 5:1 fish oil diet (5:1F). The control diet had a ratio of 8:1; however, it contained approximately half of the total PUFA provided in the other diets.

Table 4.1a: Composition of gestation sow diets (as-fed basis)

	Dietary Treatment (n-6:n-3 fatty acid ratio)				
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**
Ingredient, g/kg					
Barley	699.8	721.7	666.1	575.8	700.8
Wheat	96.0	70.0	120.0	190.0	89.0
Corn	-	-	15.0	44.0	-
Flaxseed	-	-	-	50.0	-
Soybean Meal	126.0	114.0	83.0	50.0	127.0
Canola Meal	15.5	14.0	-	-	16.0
Flaxseed Meal	-	18.0	59.8	54.0	-
Tallow	34.8	-	-	-	-
Canola Oil	-	7.2	-	-	-
Corn Oil	-	25.8	22.9	3.8	0.6
Flax Oil	-	-	5.8	4.6	-
Herring Oil	-	-	-	-	38.7
Vit-Min Premix [1]	16.0	16.0	16.0	16.0	16.0
Limestone	4.7	4.7	4.8	4.8	4.7
Dicalcium Phosphate	6.4	6.0	4.8	4.4	6.4
L-Threonine	-	-	0.3	0.4	-
L-Lysine·HCl	-	1.8	0.7	1.4	-
Choline Chloride	0.6	0.6	0.6	0.6	0.6
Ethoxyquin	0.2	0.2	0.2	0.2	0.2
Analysis					
Dry Matter, g/kg	886.7	889.5	892.1	893.6	893.5
Crude Protein, g/kg	151.8	146.6	141.7	139.5	150.3
Crude Fibre, g/kg	43.7	42.9	42.8	40.7	40.9
ADF, g/kg	58.0	59.8	66.4	58.2	52.2
Lignin, g/kg	7.4	9.8	7.3	13.0	11.3
DE[2], Mcal/kg	3.2	3.2	3.2	3.2	3.2
NE[2], Mcal/kg	2.3	2.3	2.3	2.3	2.4
SID Lysine[2], g/kg	5.4	5.4	5.4	5.4	5.4
SID Threonine[2], g/kg	4.1	4.1	4.1	4.1	4.1
SID Sulfur AA[2], g/kg	4.2	4.2	4.2	4.2	4.2
SID Tryptophan[2], g/kg	1.5	1.5	1.5	1.5	1.5
Calcium, g/kg	6.9	6.7	6.2	6.5	6.5
Total P, g/kg	4.5	4.9	4.5	4.4	4.8
Available P[2], g/kg	2.8	2.8	2.8	2.8	2.8
Crude Fat, g/kg	44.8	45.7	46.7	50.8	54.9
Total n-3, g/kg	1.8	2.9	4.9	14.2	4.8
Total n-6, g/kg	14.6	26.6	25.7	18.9	23.4
PUFA, % of total lipid	36.6	51.9	65.5	65.1	51.4
n-6:n-3 ratio	8:1	9:1	5:1	1:1	5:1

[1]Mineral premix, vitamin premix and plain salt (5.0, 6.0 and 5.0 g/kg respectively): Mineral mix provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; and Se, 0.10 mg as sodium selenite. Vitamin mix provided (per kg of diet): vitamin A, 8250 IU; vitamin D, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 25 ug.
[2]Calculated values

Table 4.1b: Fatty acid profile of gestation sow diets

	Dietary Treatment (n-6:n-3 fatty acid ratio)				
Fatty Acid, g FA/kg Diet	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**
Saturated					
Caprylic, C8:0	0.01	0.00	0.00	0.00	0.00
Capric, C10:0	0.02	0.00	0.00	0.00	0.00
Lauric, C12:0	0.03	0.01	0.01	0.01	0.03
Myristic, C14:0	0.72	0.11	0.10	0.11	1.81
Palmitic, C16:0	11.40	7.26	6.93	5.97	7.31
Stearic, C18:0	4.96	1.20	1.05	1.48	0.81
Arachidic, C20:0	0.13	0.18	0.14	0.12	0.13
Behenic, C22:0	0.02	0.02	0.03	0.05	0.13
Lignoceric, C24:0	0.05	0.08	0.07	0.08	0.06
Total Saturated	17.34	8.84	8.32	7.80	10.29
Monounsaturated					
Myristoleic, C14:1	0.07	0.03	0.02	0.03	0.10
Palmitoleic, C16:1	1.09	0.12	0.11	0.11	2.24
Oleic, C18:1 *cis*	15.14	14.26	10.43	10.16	6.13
Vaccenic, C18:1 *trans*	0.85	0.79	0.63	0.38	1.00
Eicosanoic, C20:1	0.38	0.34	0.35	0.33	5.54
Erucic, C22:1	0.06	0.02	0.02	0.02	0.04
Nervonic, C24:1	0.03	0.04	0.04	0.03	0.26
Total Monounsaturated	17.63	15.59	11.60	11.07	15.31
Polyunsaturated					
Linoleic, C18:2 n-6	14.43	26.45	25.36	18.53	13.79
γ-Linolenic, C18:3 n-6	0.04	0.02	0.04	0.08	0.05
α-Linolenic, C18:3 n-3	1.67	2.72	4.72	14.01	2.26
Eicosadienoic, C20:2 n-6	0.14	0.05	0.13	0.06	0.51
Eicosatrienoic, C20:3 n-3	0.10	0.10	0.08	0.10	0.09
Arachidonic, C20:4 n-6	0.04	0.05	0.14	0.19	9.08
Eicosapentaenoic, C20:5 n-3	0.06	0.06	0.05	0.05	1.53
Docosahexaenoic, C22:6 n-3	0.01	0.01	0.02	0.02	0.92
Total Polyunsaturated	16.49	29.46	30.55	33.04	28.23
Total n-3	1.84	2.89	4.88	14.17	4.80
Total n-6	14.65	26.57	25.67	18.87	23.43
n-6:n-3 ratio	8:1	9:1	5:1	1:1	5:1

Table 4.2a: Composition of lactation sow diets (as-fed basis)

	Dietary Treatment (n-6:n-3 fatty acid ratio)				
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**
Ingredient, g/kg					
Barley	350.0	330.0	271.0	207.0	420.0
Wheat	370.0	390.0	375.0	450.0	290.0
Corn	-	-	70.0	64.0	-
Flaxseed	-	-	-	29.0	-
Soybean Meal	194.8	160.0	129.0	91.3	184.1
Canola Meal	-	-	-	-	17.0
Flaxseed Meal	-	38.5	80.0	97.0	-
Tallow	36.5	-	-	-	-
Canola Oil	-	-	-	-	-
Corn Oil	-	31.6	24.4	3.4	1.4
Flax Oil	-	1.5	2.4	10.3	-
Herring Oil	-	-	-	-	39.0
Vit-Min Premix [1]	16.0	16.0	16.0	16.0	16.0
Limestone	8.8	8.9	8.7	8.8	8.6
Dicalcium Phosphate	18.0	17.1	16.5	15.3	18.3
L-Threonine	1.2	1.3	1.5	1.6	1.1
L-Lysine·HCl	3.9	4.3	4.7	5.5	3.7
Choline Chloride	0.6	0.6	0.6	0.6	0.6
Ethoxyquin	0.2	0.2	0.2	0.2	0.2
Analysis					
Dry Matter, g/kg	892.9	895.2	896.0	896.1	899.2
Crude Protein, g/kg	179.2	176.5	170.3	171.1	171.4
Crude Fibre, g/kg	32.9	31.8	38.7	38.7	35.1
ADF, g/kg	55.5	49.8	52.1	47.3	46.8
Lignin, g/kg	15.4	17.9	13.1	7.1	9.4
DE[2], Mcal/kg	3.28	3.28	3.29	3.29	3.28
NE[2], Mcal/kg	2.37	2.37	2.36	2.36	2.36
SID Lysine[2], g/kg	9.4	9.4	9.4	9.4	9.4
SID Threonine[2], g/kg	5.8	5.8	5.8	5.8	5.8
SID Sulfur AA[2], g/kg	4.6	4.6	4.6	4.6	4.6
SID Tryptophan[2], g/kg	1.7	1.7	1.7	1.7	1.7
Calcium, g/kg	9.4	11.1	9.7	10.0	10.2
Total P, g/kg	7.0	7.6	7.5	7.3	7.7
Available P[2], g/kg	5.0	5.0	5.0	5.0	5.0
Crude Fat, g/kg	50.9	49.9	49.9	58.6	54.5
Total n-3, g/kg	2.2	4.2	5.8	14.1	5.1
Total n-6, g/kg	15.2	29.6	27.8	18.7	23.9
PUFA, % of total lipid	34.2	67.7	67.3	56.0	53.2
n-6:n-3 ratio	7:1	7:1	5:1	1:1	5:1

[1]Mineral premix, vitamin premix and plain salt (5.0, 6.0 and 5.0 g/kg respectively): Mineral mix provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; and Se, 0.10 mg as sodium selenite. Vitamin mix provided (per kg of diet): vitamin A, 8250 IU; vitamin D, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 25 ug.

[2]Calculated values

Table 4.2b: Fatty acid profile of lactation sow diets

	Dietary Treatment (n-6:n-3 fatty acid ratio)				
Fatty Acid, g FA/kg Diet	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**
Saturated					
Caprylic, C8:0	0.01	0.00	0.00	0.00	0.00
Capric, C10:0	0.02	0.00	0.00	0.00	0.00
Lauric, C12:0	0.03	0.01	0.01	0.01	0.03
Myristic, C14:0	0.75	0.12	0.08	0.18	2.00
Palmitic, C16:0	11.36	7.43	6.64	5.70	7.21
Stearic, C18:0	5.16	1.15	1.13	1.47	0.81
Arachidic, C20:0	0.12	0.16	0.14	0.11	0.13
Behenic, C22:0	0.03	0.02	0.02	0.03	0.16
Lignoceric, C24:0	0.05	0.07	0.07	0.07	0.07
Total Saturated	17.52	8.96	8.09	7.57	10.41
Monounsaturated					
Myristoleic, C14:1	0.12	0.03	0.03	0.03	0.10
Palmitoleic, C16:1	1.10	0.14	0.10	0.24	2.22
Oleic, C18:1 *cis*	14.44	11.76	11.22	10.02	7.30
Vaccenic, C18:1 *trans*	1.23	0.67	0.59	0.37	0.17
Eicosanoic, C20:1	0.65	0.37	0.29	0.66	5.69
Erucic, C22:1	0.09	0.02	0.02	0.02	0.13
Nervonic, C24:1	0.03	0.03	0.02	0.04	0.26
Total Monounsaturated	17.66	13.02	12.28	11.36	15.87
Polyunsaturated					
Linoleic, C18:2 n-6	14.42	29.34	25.71	17.73	13.76
γ-Linolenic, C18:3 n-6	0.03	0.04	0.04	0.10	0.06
α-Linolenic, C18:3 n-3	1.73	4.05	5.69	13.84	2.57
Eicosadienoic, C20:2 n-6	0.13	0.05	0.04	0.08	0.58
Eicosatrienoic, C20:3 n-3	0.07	0.09	0.08	0.09	0.07
Arachidonic, C20:4 n-6	0.57	0.24	0.13	0.76	9.45
Eicosapentaenoic, C20:5 n-3	0.09	0.05	0.04	0.11	1.65
Docosahexaenoic, C22:6 n-3	0.07	0.03	0.02	0.06	1.03
Total Polyunsaturated	17.12	33.88	31.75	32.76	29.17
Total n-3	1.96	4.21	5.83	14.10	5.32
Total n-6	15.16	29.67	25.92	18.66	23.85
n-6:n-3 ratio	8:1	7:1	5:1	1:1	5:1

The 5:1F diet was formulated as a 1:1 ratio, however; FA analysis of the finished diet revealed that the ratio was closer to 5:1. Additionally, the 9:1P diet had a lower ratio in the lactation diet relative to the gestation diet at 7:1 n-6:n-3. Diet formulations for the gestation and lactation rations can be found in Table 4.1a/b and Table 4.2a/b.

Sows were fed 2.5 to 3.0 kg of feed per day (at 08:00) throughout breeding and gestation depending upon their body condition. Two weeks prior to farrowing their feed intake was increased to 3.0 to 3.5 kg per day. Post-farrowing, feed was provided *ad libitum* and increased daily as appetite increased.

4.3.1 Experimental Procedure

All bred and gestating sows were fed the control diet to ensure that pre-trial dietary treatments did not affect the overall results of this experiment. Sows began consuming their assigned diet five weeks pre-farrowing (± d 80 of gestation). Data was collected for two reproductive cycles on 150 sows (n=30/diet). Period 1 (P1) refers to the period from initiation of diet consumption through to weaning (approx. 7 to 8 weeks), and Period 2 (P2) refers to d 1 post-weaning to the subsequent weaning (approx. 20 to 21 weeks). The gestation diets were fed from weaning to one week pre-farrowing, and lactation diets were fed from one week pre-farrowing until weaning.

During P1, sow weights (at diet initiation and weaning), number of piglets born (total, live, stillborn, mummified), litter weights (birth and weaning), weaning to estrus interval and other production data on the animals such as cross-foster data was collected. For P2, all sows underwent more intensive data collection including measurements on average daily feed intake (ADFI) during lactation, sow weights and backfat thickness (d110 of gestation, within 24 hr post farrowing, 1 week post farrowing and again at weaning), number of piglets born (total, live, stillborn, mummified), individual piglet weights (birth and weaning) and the weaning to estrus interval. Heat checks were performed twice daily by the same technicians to ensure consistency.

During P1, sow blood samples were collected on d 110 (± 2) of gestation into evacuated collection tubes containing no additives via jugular venipunture from 12 sows

per diet. Samples were allowed to clot for 10 min and were then centrifuged at 830 × *g* for 10 min (Beckman TJ-6 Centrifuge, Beckman Coulter, Mississauga, Ontario, Canada). Serum was removed and stored at -20^{o}C until FA analysis.

During P2, serum samples were collected from 2 average BW piglets per litter, for 12 sows/diet, one piglet immediately post farrowing (pre-suckle sample) and one 24 hours post farrowing (post-suckle) via cranial vena cava venipuncture. Samples were treated as described for sows. Serum was stored at -20^{o}C until analysis of FA content, IgG and IgA.

Colostrum samples were collected from the same 12 sows/diet. A 10 to 15 ml sample was collected (composite from all functional teats) during farrowing, prior to piglets suckling, and stored at -20^{o}C until analysis. Colostrum was analyzed for FA, IgG and IgA concentrations.

Milk samples were collected from 20 randomly selected sows/diet on d 4 (early) and d 16 (late) of lactation (the same sows were used on d 4 and 16). Samples were collected following an intra-vulva injection of oxytocin (Oxyto-Sure 20 IU/ml, 1 ml; Vetoquinol, Lavaltrie, Quebec, Canada) from all functional teats after litters had been removed from the sow for 30 min. A total of 10 to 20 ml of milk was collected per sow per time period. Milk was frozen at -20^{o}C until FA analysis.

4.3.2 Analytical Methods

Proximate analysis of diets was performed by Central Testing Laboratory Ltd (Winnipeg, Manitoba, Canada). Measures included DM (method 930.15; AOAC, 1990), ash (method 923.03; AOAC, 1990), N (Leco Analyzer, St. Joseph, MI), crude fat (ANKOM XT20), crude fibre (AOCS Ba6a-05), ADF (ANKOM 08-16-16), lignin (ANKOM 3/98), Ca and P (methods 968.08 and 935.13A; AOAC, 1990).

Diets were analyzed for their FA profile using gas liquid chromatography (GLC, Agilent 6890 system with Agilent ChemStation Software; Agilent Technologies, Mississauga, Ontario, Canada). Direct FA methylation was performed according to the procedure of O'Fallon et al. (2007). Non-methylated C13:0 (Nu-Chek Prep Inc, Elysian,

MN) was used as an internal standard, and all other chemicals used were of GLC grade (Sigma-Aldrich Inc., St. Louis, MO). Fatty acid methyl ester (FAME) samples were compared with a standard mixture containing a wide array of FAME's ranging from C8:0 to C24:1 (GLC-68-D, GLC-97 and U-62-M; Nu-Chek Prep Inc, Elysian, MN) using a GLC program slightly modified from the procedure described by O'Fallon et al. (2007). Briefly, the instrument was set for a 1.0 µl injection and split at a ratio of 30:1. The injector set points were a temperature of 260^{o}C, pressure of 40.24 psi, and a total flow for the carrier gas (helium) of 37.5 mL/min. The initial oven temperature was 140^{o}C and held for 5 min. The temperature was ramped up at a rate of 4^{o}C/min to a maximum of 240^{o}C and held for 15 min. The total run time for analysis was 45 min. A Supelco fused silica capillary SP 2560 column (Sigma-Aldrich Inc., St. Louis, MO) was used for analysis. A flame ionization detector was utilized for detection, set at 250^{o}C, hydrogen flow of 40 mL/min, air flow of 450 mL/min and helium flow of 45 mL/min.

Serum, colostrum and milk samples were also analyzed for their FA profile using GLC. Direct FA methylation was performed according to the procedure of Gao et al. (2009), which is a modification for smaller sample sizes of the direct FAME extraction described by O'Fallon et al. (2007). Non-methylated C13:0 (Nu-Chek Prep Inc, Elysian, MN) was used as the internal standard, and all other chemicals were of GLC grade (Sigma-Aldrich Inc., St. Louis, MO). Fatty acid methyl ester samples were compared with a standard mixture containing a wide array of FAME's ranging from C8:0 to C24:1 (GLC-68-D, GLC-97 and U-62-M; Nu-Chek Prep Inc, Elysian, MN). The GLC program was set for a 1.0 µl injection and split at a ratio of 20:1. The injector set points were a temperature of 250^{o}C, pressure of 15.47 psi, and a total flow for the carrier gas (helium) of 23.3 mL/min. The initial oven temperature was 130^{o}C and held for 1 min. The temperature was ramped up at a rate of 6.5^{o}C/min to 170^{o}C, followed by 2.75^{o}C/min to 215^{o}C and held for 12 min, then 5^{o}C/min to 230^{o}C with a 3 min hold and finally 5^{o}C/min to a max temp of 240^{o}C with a 10 min hold. The total run time was 54 min. A DB-23 fused silica capillary column (Agilent Technologies, Mississauga, Ontario, Canada) was used for analysis and an flame ionization detector was utilized for detection, with the heater set at 250^{o}C, hydrogen flow of 40 mL/min, air flow of 350 mL/min and helium flow of 35 mL/min.

Colostrum and piglet serum samples were analyzed for their IgA and IgG concentrations by Prairie Diagnostic Services (Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada) using commercial ELISA kits (Bethyl Laboratories, Inc., Montgomery, TX) according to manufacturers' specifications. Limits of detection were 0.1 mg/ml for IgA and 0.9 mg/ml for IgG. Antibodies included in the kits react only with the specific pig Ig (such as IgG), not with other porcine Ig's or serum proteins.

4.3.3 Calculations & Statistics

The serum long chain n-3 (sLC) to dietary ALA intake (ALAin) ratio (sLC:ALAin) was calculated to allow estimation of the difference in conversion efficiency of dietary ALA into its longer chain counterparts across treatment groups (Welch et al., 2010). This calculation was conducted for sows and their offspring (pre and post suckle). The sLC value was calculated as the sum of serum eicosatrienoic acid (ETA), EPA and DHA (in mg FA/ml serum). ALA intake was calculated differently for sows and piglets. For sows, the intake of ALA (mg FA/d) was determined by multiplying the amount of ALA in the diet (mg FA/g diet) by the gestation ADFI (g/d). For pre-suckle piglets, sow ALA intake values were used for ALAin. For post-suckle piglets (24 hours post farrowing), ALAin was estimated as the amount of ALA in colostrum multiplied by 300 g colostrum intake per piglet. The literature reports average colostrum intakes from 290 g to 490 g per pig (Herpin and Le Dividich, 1995), with more recent averages of 300 g in a 24 hour period (Devillers et al., 2007). A second set of ratio calculations were also included, the ratio of serum EPA (sEPA) to ALAin (sEPA:ALAin), since conversion of ALA to EPA is generally more efficient than the conversion to DHA. Calculation of ALAin was the same as that described above.

Data was analyzed using the Mixed Model of SAS (version 9.2; SAS Inst. Inc., Cary, NC) for a completely randomized design. Initially, the model included parity as a block; however, parity was non-significant for any measure and thus was removed from the model. For all parameters sow was considered the random effect and diet a fixed effect. Tukey's honestly significant difference was used for means separation when a main effect was present. Stillbirths, mummies and total numbers of piglets born dead

were analyzed using a Poisson regression distribution within the Genmod procedure, with estimate statements to compare dietary treatments. Probability values of ≤ 0.05 were considered significant; values between 0.05 and 0.10 were considered a trend, and those > 0.10 were non-significant.

4.4 Results

Thirteen sows had to be replaced during P1 and 17 in P2. No specific treatment group was overly represented by sows that were lost or removed from trial. Reasons for removal included abortion (1), prolapse (2), sold (2), heart attack (2), difficulty farrowing or milking (2), lame (6), failure to rebreed (7) and generalized ill-health (8).

The average daily intakes of LA, ALA, ArA, EPA, DHA, total MUFA, and total PUFA for sows are shown in Table 4.3. Intake of ALA increased as the dietary ratio decreased, and intake of LA decreased with decreasing ratio. Eicosapentaenoic acid and DHA intakes were highest in the fish based diet. Arachidonic acid intake was also highest in the fish based diet. The fish oil source contained greater quantities of ArA than previously reported in the literature.

Performance results for P1 and P2 are shown in Table 4.4. Dietary treatment had no effect on the total number of piglets born, born alive, litter birth weights or weaning to estrus intervals for either period ($P > 0.05$). In P1, average piglet weaning weight was higher for piglets raised by sows consuming the 9:1P and 5:1P diet compared to those raised by sows consuming the 1:1P or 5:1F, with piglets raised by control sows having intermediate weaning weights ($P = 0.02$). In P2, piglets from sows consuming the 5:1F diet had reduced birth weights ($P = 0.05$), and average weaning weight was reduced by 0.8 kg/piglet ($P = 0.04$) compared to piglets raised by sows consuming the control and 5:1P diets. Additionally, 5:1F fed sows consumed 10% less feed ($P = 0.04$) when compared to sows consuming the control or 5:1P diets. Pre-weaning mortality was unaffected by diet during P1 ($P > 0.05$); however, the rate was lower in the 9:1P and 1:1P groups and higher in the 5:1P and 5:1F groups relative to the control diet group during P2 ($P < 0.01$). Conception rates, defined as the percentage of sows who conceived upon first breeding (did not return to estrus), were 100%, 84%, 94% 93% and 97% at the start of P2

for the control, 9:1P, 5:1P, 1:1P and 5:1F diets respectively and were 89%, 100%, 97%, 96% and 97% in the breeding period immediately following P2.

Poisson regression analysis showed that as the n-6:n-3 FA ratio decreased in sow diets, the number of stillbirths and mummies increased. During P1 (Figure 4.1), there was a numerical linear increase in the number of stillbirths, mummies and total born dead as the n6:n3 ratio decreased, however this was not significant. In P2 (Figure 4.2), similar patterns existed, with the numbers of piglets born dead increasing as the n-6:n-3 ratio decreased. In this period however, the effect was significant for the number of stillbirths ($P = 0.02$) and total born dead ($P = 0.02$), but not for mummified piglets, with significantly more stillbirths and total born dead in the 9:1P, 5:1P, 1:1P and 5:1F diets relative to the control diet. Additionally, there was a tendency for increased numbers of stillborn piglets in the 1:1P diet relative to the 9:1P and 5:1P diets ($P = 0.1$).

Immunoglobulin (IgA and IgG) concentrations measured in colostrum and piglet serum (pre and post suckle) are shown in Table 4.5. There were no effects of sow diet composition on Ig concentrations in colostrum or serum ($P > 0.05$). Immunoglobulin concentration was below the limit of detection for pre-suckle piglet serum samples.

The FA profile of colostrum collected at the time of farrowing is shown in Table 4.6. In general, the profile pattern was similar to that of the respective sow dietary FA profile. A decrease in the dietary n-6:n-3 ratio was reflected in the colostrum. The ratios were lower however, in the colostrum than the diets. For example, the 9:1, 5:1 and 1:1 plant based diets provided a colostrum profile of 5:1, 4:1 and 1:1 respectively. Also, colostrum from sows fed the fish based diet had lower amounts of total n-3 FA's when compared to the colostrum produced from sows fed the plant based diets, and the n-3 composition was different (ALA for plant based sows, EPA/DHA for fish based sows). Similar FA patterns were observed for early lactation milk samples (Table 4.7) and late lactation milk samples (Table 4.8).

Table 4.3: Average daily fatty acid intakes in gestation and lactation during period 2

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics[3]	
Fatty Acid, g FA/d	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	**P Value**
			Gestation[1]				
Number of Sows	30	28	31	30	30		
Linoleic Acid (18:2 n-6)	36.08	66.11	63.40	46.32	34.46	-	-
α-Linolenic Acid (18:3 n-3)	4.18	6.79	11.80	35.02	5.66	-	-
Arachidonic Acid (20:4 n-6)	0.11	0.14	0.35	0.48	22.70	-	-
Eicosapentaenoic Acid (20:5 n-3)	0.14	0.16	0.14	0.12	3.83	-	-
Docosahexaenoic Acid (22:6 n-3)	0.03	0.01	0.04	0.05	2.30	-	-
Total n-3	4.59	7.22	12.19	35.43	12.01	-	-
Total n-6	36.64	66.42	64.18	47.16	58.57	-	-
Total Saturated Fatty Acid	43.34	22.11	20.81	19.51	25.72	-	-
Total Monounsaturated Fatty Acid	44.07	38.98	29.01	27.68	38.27	-	-
Total Polyunsaturated Fatty Acid	41.23	73.64	76.37	82.60	70.57	-	-
			Lactation[2]				
Number of Sows	30	28	31	30	30		
Linoleic Acid (18:2 n-6)	108.21^{a}	217.25^{c}	208.52^{c}	133.63^{b}	93.56^{a}	4.476	< 0.01
α-Linolenic Acid (18:3 n-3)	14.78^{a}	29.96^{b}	43.22^{c}	104.35^{d}	16.12^{a}	1.487	< 0.01
Arachidonic Acid (20:4 n-6)	4.30^{ab}	1.75^{a}	2.10^{a}	5.70^{b}	64.26^{c}	0.851	< 0.01
Eicosapentaenoic Acid (20:5 n-3)	0.68^{a}	0.34^{a}	0.29^{a}	0.85^{a}	10.92^{b}	0.144	< 0.01
Docosahexaenoic Acid (22:6 n-3)	0.45^{a}	0.22^{a}	0.13^{a}	0.43^{a}	6.99^{b}	0.092	< 0.01
Total n-3	16.57^{a}	31.17^{b}	44.24^{c}	106.31^{d}	34.59^{b}	1.571	< 0.01
Total n-6	113.77^{a}	219.67^{b}	211.23^{b}	140.63^{c}	162.16^{d}	4.898	< 0.01
Total Saturated Fatty Acid	130.17^{a}	66.31^{bc}	61.41^{cd}	57.02^{d}	70.76^{b}	2.093	< 0.01
Total Monounsaturated Fatty Acid	123.71^{a}	96.39^{b}	93.21^{b}	85.85^{b}	123.71^{c}	2.716	< 0.01
Total Polyunsaturated Fatty Acid	129.53^{a}	250.84^{b}	241.00^{b}	246.94^{b}	250.84^{c}	6.104	< 0.01

[a-d]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]All gestating sows were fed 2.5 kg of feed per day until 2 weeks pre-farrowing where intakes were increased to 3.0 kg of feed per day. Calculations are based on 2.5 kg intakes.

[2]Based on average daily feed intake values of 7.5, 7.4, 7.6, 7.5 and 6.8 kg per day for the control, 9:1P, 5:1P, 1:1P and 5:1F diets respectively

[3]No statistics are presented for gestation intakes as all sows consumed the same amount of feed and thus no variation exists among sows within a diet treatment group in FA intakes

Table 4.4: Reproductive performance of sows consuming differing dietary n-6 to n-3 ratios during periods 1 and 2

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	**P Value**
Period 1[1]							
Number of Sows	31	31	33	33	30	-	-
Sow Weight, gestation d 110[2], kg	235.5	245.9	244.8	236.8	238.8	5.85	0.62
Sow Weight, Weaning, kg	233.1	244.5	243.6	248.1	237.1	5.76	0.35
Avg. No. Born Total	13.7	13.6	13.7	14.3	14.4	0.54	0.73
Avg. No. Born Alive	12.8	12.6	12.4	13.0	13.0	0.50	0.92
Avg. No. Weaned[3]	10.7	10.3	10.5	10.3	10.7	0.28	0.38
Pre-weaning Mortality[4]	10.6	15.5	15.2	18.3	16.8	2.46	0.25
Live Litter Birth Weight, kg	18.7	18.3	18.5	17.9	17.7	0.78	0.89
Total Litter Birth Weight, kg	19.6	19.5	19.7	19.3	18.8	0.77	0.93
Avg. Piglet Birth Weight, kg	1.5	1.5	1.5	1.4	1.3	0.05	0.10
Total Litter Weaning Weight[3], kg	87.2	88.3	90.8	80.2	83.6	2.86	0.07
Avg. Piglet Weaning Weight[3], kg	8.2^{ab}	8.6^{a}	8.6^{a}	8.0^{b}	7.8^{b}	0.19	0.02
Weaning to Estrus Interval, d	5.2	4.9	4.8	4.6	4.6	0.46	0.87
Period 2[1]							
Number of Sows	30	28	31	30	30	-	-
Sow Weight, d 110[2], kg	265.9	279.6	277.6	278.8	274.6	4.82	0.26
Sow Weight, Farrowing, kg	254.2	266.0	270.0	265.6	263.9	5.17	0.26
Sow Weight, d 7[2], kg	255.4	267.4	268.8	267.1	263.6	5.35	0.41
Sow Weight, Weaning, kg	248.6	260.5	264.4	262.9	252.8	5.40	0.17
Total Weight Change, kg/lact	-5.6	-8.0	-5.6	-3.3	-11.7	2.63	0.29
Avg. Daily Weight Change, kg/d	-0.2	-0.3	-0.2	-0.1	-0.4	0.10	0.34
Backfat Thickness, d 110[2], mm	13.6	14.3	14.5	14.8	14.2	0.35	0.15
Backfat Thickness, Farrowing, mm	12.9	13.7	13.8	14.1	13.6	0.34	0.18
Backfat Thickness d 7[2], mm	12.5^{b}	13.4^{ab}	13.7^{a}	14.1^{a}	13.4^{ab}	0.33	0.02
Backfat Thickness Weaning, mm	11.9^{b}	12.6^{ab}	13.1^{a}	13.2^{a}	12.9^{a}	0.33	0.04
Total Backfat Change, mm/lact	-0.8	-1.1	-0.7	-0.9	-0.7	0.22	0.71
Avg. Daily Backfat Change, mm/d	-0.03	-0.04	-0.02	-0.03	-0.03	0.009	0.83
Sow Lactation ADFI, kg/d	7.5^{a}	7.4^{a}	7.6^{a}	7.5^{a}	6.8^{b}	0.20	0.04
Avg. No. Born Total	13.3	14.0	12.9	14.0	14.4	0.63	0.46
Avg. No. Born Alive	12.5	12.5	11.5	12.3	13.0	0.60	0.54
Avg. No. Weaned[3]	10.1^{ab}	10.4^{a}	10.0^{ab}	9.5^{b}	9.3^{b}	0.27	0.04
Pre-weaning Mortality[4]	16.2^{ab}	11.1^{a}	22.7^{bc}	13.2^{a}	24.0^{c}	2.44	< 0.01
Live Litter Birth Weight, kg	18.1	17.5	16.8	17.7	16.9	0.77	0.72
Total Litter Birth Weight, kg	18.9	19.0	18.0	19.8	18.4	0.79	0.62
Avg. Piglet Birth Weight, kg	1.5^{a}	1.4^{ab}	1.5^{a}	1.4^{ab}	1.3^{b}	0.05	0.05
Total Litter Weaning Weight[3], kg	88.7^{a}	88.6^{a}	90.4^{a}	83.0^{ab}	77.0^{b}	2.88	< 0.01
Avg. Piglet Weaning Weight[3], kg	8.8^{a}	8.7^{ab}	9.2^{a}	8.7^{ab}	8.2^{b}	0.21	0.04
Piglet ADG, kg/d	0.31^{a}	0.30^{ab}	0.32^{a}	0.30^{ab}	0.29^{b}	0.007	0.04
Weaning to Estrus Interval, d	4.1	4.9	4.2	3.9	5.1	0.42	0.17

[a-b]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]Periods 1 and 2 refer to the reproductive cycle of the sows since initiating diet consumption. Period 1 is the farrowing and lactation period immediately following the start of diet consumption on d 80 of gestation; Period 2 is the subsequent breeding to weaning period

[2]d 110 refers to the 110th day of gestation, d 7 refers to the 7th day of lactation

[3]Piglet weaning data includes any cross-fostered piglets

[4]Pre-weaning mortality rate is expressed as a % of all piglets nursing within a litter

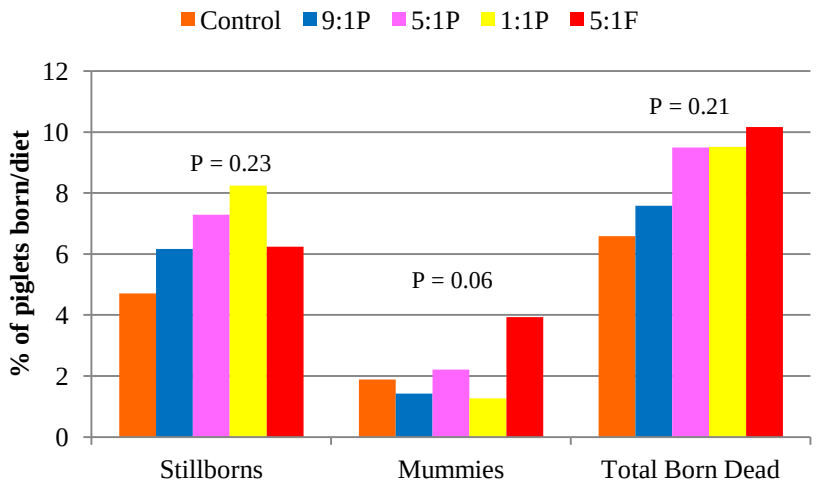

Figure 4.1: Total number of stillbirths, mummies and total born dead during period 1, expressed as % of piglets born per diet, analyzed using Poisson regression distribution.

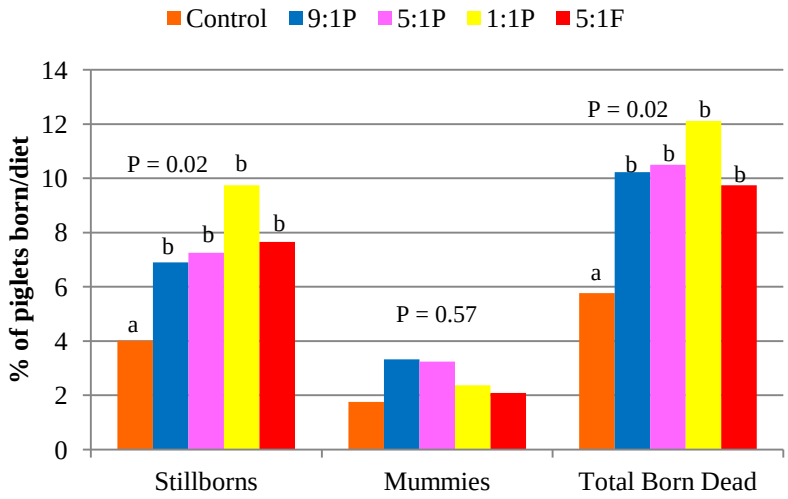

Figure 4.2: Total number of stillbirths, mummies and total born dead during period 2, expressed as % of piglets born per diet, analyzed using Poisson regression distribution. Within a group, bars without common superscripts differ ($P \leq 0.05$)

Table 4.5: IgA and IgG concentrations (mg/ml) in colostrum and piglet pre- and post-suckle serum samples (n = 12/diet)

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	**P Value**
Colostrum IgA	16.4	18.3	15.8	17.6	15.6	1.95	0.84
Pre-suckle IgA	ND[1]	ND	ND	ND	ND	-	-
Post-suckle IgA	7.4	6.5	7.7	7.1	6.8	1.26	0.96
Colostrum IgG	82.8	90.7	81.4	87.3	82.9	7.55	0.90
Pre-suckle IgG	ND	ND	ND	ND	ND	-	-
Post-suckle IgG	27.9	23.3	25.9	26.9	24.1	4.17	0.93

[1]ND = not detected, assay detection limits were 0.1 mg/ml for IgA and 0.9 mg/ml for IgG

The FA profile of sow plasma is shown in Table 4.9. Total plasma n-3 FA's were greater in sows ($P < 0.01$) consuming the 1:1P and 5:1F diets, and in serum from their piglets post-suckle ($P < 0.01$). The ALA content was highest in the 1:1P sows whereas EPA and DHA were highest in the 5:1F sows. ALA, EPA, DHA and total n-3's are depicted in Figure 4.3.

Pre-suckle piglet plasma FA's are detailed in Table 4.10 and Figure 4.4. In pre-suckle piglet plasma, ALA and DHA did not differ among treatment groups ($P > 0.05$). Relative to the piglets raised by sows consuming the control diet, EPA was 2.5 times greater in piglets raised by sows consuming the 1:1P diet and 4 times greater in sows fed the 5:1F diet ($P < 0.01$).

In post-suckle serum samples (Table 4.11 and Figure 4.5) ALA was highest in piglets produced by sows consuming the 1:1P diet ($P < 0.01$) while EPA and DHA were highest in serum from piglets from the 5:1F fed sows ($P < 0.01$).

An estimation of conversion efficiency across dietary treatments is presented in Table 4.12. The sLC:ALAin and sEPA:ALAin ratios were highest in the sows fed the 5:1F treatment. Both ratios were non-significant for post-suckle piglets, and there were no differences between the three plant based treatments for sows or pre-suckle piglets.

Table 4.6: Fatty acid profile of colostrum samples[1] collected during farrowing[2]

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
Fatty Acid, mg FA/ml colostrum	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Saturated							
Caprylic, C8:0	0.47	0.44	0.95	1.19	1.08	0.263	0.15
Capric, C10:0	0.03	0.03	0.05	0.04	0.03	0.006	0.14
Lauric, C12:0	0.14	0.11	0.13	0.13	0.13	0.019	0.92
Myristic, C14:0	6.39^{ab}	4.41^{b}	4.47^{b}	5.03^{b}	7.74^{a}	0.732	0.01
Palmitic, C16:0	61.77	57.24	49.18	57.71	43.03	6.299	0.28
Stearic, C18:0	13.43	12.28	18.99	15.55	15.53	3.039	0.59
Arachidic, C20:0	0.54	0.49	0.41	0.42	1.14	0.199	0.10
Behenic, C22:0	0.20^{a}	0.22^{a}	0.23^{a}	0.78^{a}	2.39^{b}	0.227	<0.01
Lignoceric, C24:0	1.55^{a}	1.71^{a}	1.73^{a}	3.05^{b}	3.16^{b}	0.271	<0.01
Monounsaturated							
Myristoleic, C14:1	0.14	0.11	0.09	0.15	0.12	0.020	0.30
Palmitoleic, C16:1	9.04^{ab}	5.42^{c}	5.40^{c}	6.98^{bc}	10.25^{a}	1.075	0.01
Oleic, C18:1 *cis*	85.65^{a}	78.67^{a}	47.46^{bc}	76.68^{ab}	39.87^{c}	10.400	0.01
Vaccenic, C18:1 *trans*	6.57	5.11	3.01	8.78	7.01	1.668	0.17
Eicosanoic, C20:1	0.49^{a}	0.70^{a}	0.39^{a}	0.56^{a}	1.55^{b}	0.201	<0.01
Erucic, C22:1	0.28^{a}	0.32^{a}	0.37^{a}	1.54^{b}	2.06^{b}	0.346	0.01
Nervonic, C24:1	0.24^{a}	0.23^{a}	0.22^{a}	0.24^{a}	0.65^{b}	0.039	<0.01
Polyunsaturated							
Linoleic, C18:2 n-6	44.90^{a}	96.47^{c}	81.88^{bc}	70.04^{b}	28.40^{a}	8.474	<0.01
γ-Linolenic, C18:3 n-6	0.39	0.66	0.57	0.47	0.42	0.081	0.13
α-Linolenic, C18:3 n-3	4.54^{a}	16.43^{a}	17.31^{a}	50.38^{b}	4.85^{a}	4.683	<0.01
Eicosadienoic, C20:2 n-6	0.78^{a}	1.39^{a}	1.22^{a}	1.26^{a}	3.08^{b}	0.390	<0.01
Eicosatrienoic, C20:3 n-3	1.50	1.85	1.45	2.01	0.79	0.336	0.16
Arachidonic, C20:4 n-6	1.18^{ab}	1.57^{a}	0.88^{bc}	1.07^{bc}	0.59^{c}	0.163	<0.01
Eicosapentaenoic, C20:5 n-3	0.71^{a}	0.80^{a}	0.75^{a}	1.93^{b}	1.86^{b}	0.304	<0.01
Docosahexaenoic, C22:6 n-3	0.53^{a}	0.45^{a}	0.38^{a}	0.68^{a}	5.33^{b}	0.346	<0.01
Total n-3	7.29^{a}	19.53^{a}	19.89^{a}	55.01^{b}	12.83^{a}	4.959	<0.01
Total n-6	47.24^{a}	100.08^{b}	84.54^{bc}	72.85^{c}	32.49^{a}	8.805	<0.01
n-6:n-3 ratio	6.5:1	5.1:1	4.3:1	1.3:1	2.5:1	-	-

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]12 sows per diet

[2]Colostrum collection began when the sow started farrowing until a 10 to 15 ml representative sample was obtained

Table 4.7: Fatty acid profile of early[1] lactation milk samples[2]

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
Fatty Acid, mg FA/ml Milk	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Saturated							
Caprylic, C8:0	1.10	0.87	0.91	1.13	1.15	0.199	0.78
Capric, C10:0	0.79	0.65	0.74	0.57	0.53	0.099	0.34
Lauric, C12:0	0.85	0.65	0.76	0.77	0.73	0.102	0.73
Myristic, C14:0	12.32	8.64	10.16	11.85	15.07	1.542	0.08
Palmitic, C16:0	115.49	98.93	109.18	117.60	133.77	11.104	0.30
Stearic, C18:0	19.44	14.92	16.11	20.76	27.58	3.796	0.18
Arachidic, C20:0	1.03^{a}	1.04^{a}	0.89^{a}	0.92^{a}	5.27^{b}	0.402	<0.01
Behenic, C22:0	0.23^{a}	0.13^{a}	0.10^{a}	0.35^{a}	5.72^{b}	0.398	<0.01
Lignoceric, C24:0	1.13^{a}	1.06^{a}	1.45^{a}	3.21^{b}	5.34^{c}	0.533	<0.01
Monounsaturated							
Myristoleic, C14:1	0.98^{a}	0.58^{c}	0.70^{bc}	0.73^{abc}	0.97^{ab}	0.093	0.02
Palmitoleic, C16:1	38.65	29.45	34.33	32.84	38.06	3.587	0.37
Oleic, C18:1 *cis*	116.77	75.42	90.22	111.90	166.18	23.668	0.11
Vaccenic, C18:1 *trans*	8.05^{a}	4.45^{a}	5.37^{a}	7.28^{a}	14.42^{b}	1.768	<0.01
Eicosanoic, C20:1	0.19^{a}	0.23^{a}	0.42^{a}	0.36^{a}	10.61^{b}	0.732	<0.01
Erucic, C22:1	0.26^{a}	0.20^{a}	0.16^{a}	0.30^{a}	2.33^{b}	0.407	<0.01
Nervonic, C24:1	0.17^{a}	0.12^{a}	0.11^{a}	0.27^{a}	0.70^{b}	0.082	<0.01
Polyunsaturated							
Linoleic, C18:2 n-6	39.64	69.14	73.30	75.89	64.50	10.703	0.16
γ-Linolenic, C18:3 n-6	0.56	0.54	0.60	0.60	0.58	0.140	0.99
α-Linolenic, C18:3 n-3	3.86^{a}	8.50^{a}	14.22^{a}	49.95^{b}	8.32^{a}	4.292	<0.01
Eicosadienoic, C20:2 n-6	1.04	0.92	1.02	1.48	1.02	0.338	0.80
Eicosatrienoic, C20:3 n-3	0.22^{a}	0.32^{a}	0.51^{a}	2.28^{b}	0.67^{a}	0.225	<0.01
Arachidonic, C20:4 n-6	2.10	1.45	1.52	1.61	1.72	0.386	0.77
Eicosapentaenoic, C20:5 n-3	0.68^{a}	0.29^{a}	0.52^{a}	2.39^{ab}	3.99^{b}	0.721	<0.01
Docosahexaenoic, C22:6 n-3	0.38^{a}	0.23^{a}	0.30^{a}	0.97^{a}	7.23^{b}	0.622	<0.01
Total n-3	5.15^{a}	9.33^{ac}	15.55^{ac}	55.59^{b}	20.21^{c}	5.103	<0.01
Total n-6	43.34	72.06	76.44	79.58	67.82	11.332	0.21
n-6:n-3 ratio	8.4:1	7.7:1	4.9:1	1.4:1	3.4:1	-	-

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]Samples were collected on d 4 of lactation following an intra-vulva injection of oxytocin (Oxyto-Sure 20 IU/ml, 1 ml; Vetoquinol, Lavaltrie, Quebec, Canada) from all functional teats after litters had been removed from the sow for 30 min.

[2]5 sows per diet

Table 4.8: Fatty acid profile of late[1] lactation milk samples[2]

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
Fatty Acid, mg FA/ml Milk	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Saturated							
Caprylic, C8:0	1.00	1.32	0.83	1.07	1.06	0.242	0.72
Capric, C10:0	1.41	1.27	1.48	1.37	1.20	0.138	0.63
Lauric, C12:0	1.53	1.39	1.62	1.57	1.47	0.115	0.64
Myristic, C14:0	19.08	16.30	19.40	18.68	20.90	1.276	0.19
Palmitic, C16:0	160.05	149.21	177.37	113.82	142.05	15.859	0.11
Stearic, C18:0	19.84	16.85	20.97	22.76	16.23	3.826	0.72
Arachidic, C20:0	1.23	0.92	1.19	7.40	4.68	3.080	0.50
Behenic, C22:0	0.23^{a}	0.16^{a}	0.16^{a}	0.63^{a}	4.18^{b}	0.385	<0.01
Lignoceric, C24:0	0.75^{a}	0.92^{ac}	1.26^{bc}	1.45^{b}	2.88^{d}	0.154	<0.01
Monounsaturated							
Myristoleic, C14:1	1.70	1.20	1.36	1.55	1.19	0.165	0.16
Palmitoleic, C16:1	53.85	43.72	53.12	72.44	47.05	11.043	0.42
Oleic, C18:1 *cis*	125.48	107.83	130.40	77.22	118.01	16.725	0.21
Vaccenic, C18:1 *trans*	7.94^{ab}	6.39^{b}	7.81^{ab}	4.87^{b}	9.89^{a}	1.060	0.04
Eicosanoic, C20:1	0.21^{a}	0.42^{a}	0.19^{a}	0.45^{a}	9.51^{b}	0.408	<0.01
Erucic, C22:1	0.24^{a}	0.27^{a}	0.22^{a}	0.72^{ab}	1.55^{b}	0.284	0.02
Nervonic, C24:1	0.16^{a}	0.19^{a}	0.17^{a}	0.18^{a}	0.49^{b}	0.047	0.01
Polyunsaturated							
Linoleic, C18:2 n-6	42.36^{a}	82.14^{b}	83.08^{b}	44.69^{a}	45.87^{a}	6.528	<0.01
γ-Linolenic, C18:3 n-6	0.30	0.24	0.30	19.12	0.30	8.474	0.44
α-Linolenic, C18:3 n-3	4.52^{a}	10.34^{ab}	17.21^{b}	40.72^{c}	6.46^{a}	2.379	<0.01
Eicosadienoic, C20:2 n-6	0.88^{bc}	1.18^{ab}	1.52^{a}	0.64^{bc}	0.56^{c}	0.195	0.01
Eicosatrienoic, C20:3 n-3	0.35^{a}	0.26^{a}	0.90^{b}	1.08^{b}	0.62^{ab}	0.177	0.02
Arachidonic, C20:4 n-6	1.33	1.23	1.46	1.17	0.91	0.238	0.57
Eicosapentaenoic, C20:5 n-3	0.78^{a}	0.54^{a}	0.51^{a}	1.34^{a}	4.09^{b}	0.451	<0.01
Docosahexaenoic, C22:6 n-3	0.31^{a}	0.22^{a}	0.19^{a}	0.66^{b}	4.76^{c}	0.118	<0.01
Total n-3	5.95^{a}	11.36^{ab}	18.81^{b}	43.80^{c}	15.94^{b}	2.615	<0.01
Total n-6	44.87^{a}	84.79^{b}	86.35^{b}	65.62^{ab}	47.65^{a}	7.992	<0.01
n-6:n-3 ratio	7.5:1	7.5:1	4.6:1	1.5:1	3.0:1	-	-

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]Samples were collected on d 16 of lactation following an intra-vulva injection of oxytocin (Oxyto-Sure 20 IU/ml, 1 ml; Vetoquinol, Lavaltrie, Quebec, Canada) from all functional teats after litters had been removed from the sow for 30 min.

[2]5 sows per diet

Table 4.9: Fatty acid profile of sow serum collected on d 110 (± 2) of gestation[1]

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
Fatty Acid, mg FA/ml Serum	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Saturated							
Caprylic, C8:0	0.18	0.17	0.31	0.20	0.26	0.057	0.38
Capric, C10:0	ND[2]	ND	ND	ND	ND	-	-
Lauric, C12:0	<0.01	<0.01	<0.01	<0.01	<0.01	0.001	0.32
Myristic, C14:0	0.06^{a}	0.04^{ab}	0.03^{b}	0.03^{b}	0.12^{c}	0.005	<0.01
Palmitic, C16:0	1.00^{ab}	0.91^{ab}	0.78^{b}	0.81^{ab}	1.05^{a}	0.062	0.01
Stearic, C18:0	0.72	0.75	0.68	0.71	0.55	0.059	0.17
Arachidic, C20:0	0.01	0.01	0.01	0.01	0.04	0.011	0.31
Behenic, C22:0	<0.01^{a}	<0.01^{a}	<0.01^{a}	<0.01^{a}	0.20^{b}	0.020	<0.01
Lignoceric, C24:0	0.06	0.05	0.08	0.06	0.08	0.015	0.46
Monounsaturated							
Myristoleic, C14:1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.001	0.67
Palmitoleic, C16:1	0.07^{a}	0.03^{b}	0.03^{b}	0.03^{b}	0.15^{c}	0.006	<0.01
Oleic, C18:1 *cis*	1.11^{a}	0.86^{ab}	0.66^{ab}	0.66^{ab}	0.61^{b}	0.114	0.01
Vaccenic, C18:1 *trans*	0.09	0.07	0.05	0.05	0.21	0.055	0.19
Eicosanoic, C20:1	0.02^{a}	0.02^{a}	0.02^{a}	0.01^{a}	0.17^{b}	0.013	<0.01
Erucic, C22:1	0.02	0.02	0.04	0.03	0.04	0.010	0.36
Nervonic, C24:1	0.03	0.02	0.05	0.03	0.05	0.008	0.06
Polyunsaturated							
Linoleic, C18:2 n-6	1.20	1.56	1.39	1.12	1.18	0.211	0.60
γ-Linolenic, C18:3 n-6	0.02	0.04	0.08	0.17	0.03	0.042	0.08
α-Linolenic, C18:3 n-3	0.06^{a}	0.09^{a}	0.17^{a}	0.37^{b}	0.10^{a}	0.042	<0.01
Eicosadienoic, C20:2 n-6	0.02^{ab}	0.03^{a}	0.02^{abc}	0.02^{bc}	0.02^{c}	0.001	<0.01
Eicosatrienoic, C20:3 n-3	0.01^{a}	0.01^{a}	0.02^{ab}	0.06^{ab}	0.09^{b}	0.016	<0.01
Arachidonic, C20:4 n-6	0.20^{a}	0.19^{a}	0.14^{ab}	0.09^{b}	0.11^{b}	0.023	<0.01
Eicosapentaenoic, C20:5 n-3	0.02^{ab}	0.02^{a}	0.03^{ab}	0.06^{b}	0.26^{c}	0.015	<0.01
Docosahexaenoic, C22:6 n-3	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.10^{b}	0.009	<0.01
Total n-3	0.11^{a}	0.14^{a}	0.24^{a}	0.51^{b}	0.55^{b}	0.050	<0.01
Total n-6	1.44	1.82	1.63	1.40	1.34	0.202	0.46
n-6:n-3 ratio	13.1:1	13.0:1	6.8:1	2.7:1	2.4:1	-	-

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]Serum was collected from 12 sows per diet 30 days after diet consumption began

[2]ND = not detected, limit of detection was 0.005 mg FA/ml plasma

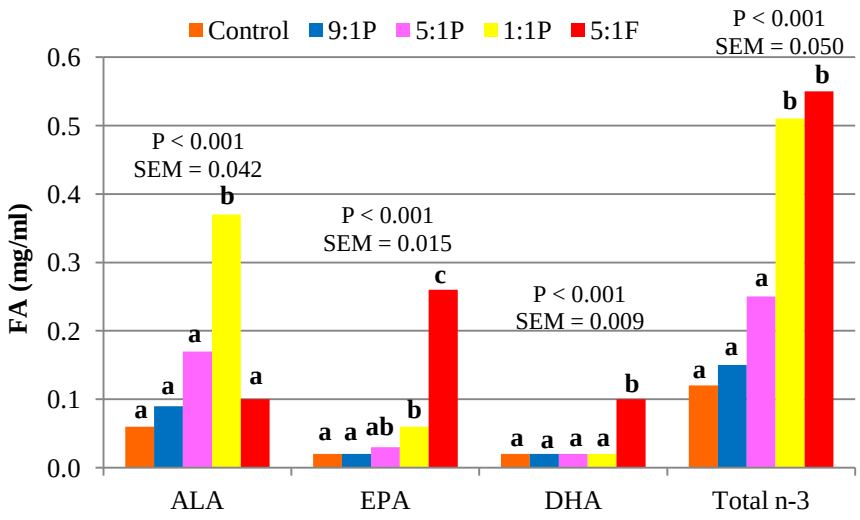

Figure 4.3: Sow serum α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and total n-3 profiles collected on d 110 of gestation (n=12/diet). Treatments refer to the tallow based control and the n6:n3 ratio. Bars within each fatty acid group without common superscripts differ ($P \leq 0.05$).

Table 4.10: Fatty acid profile of pre-suckle piglet serum collected at farrowing[1]

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
Fatty Acid, mg FA/ml Serum	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Saturated							
Caprylic, C8:0	0.06	0.04	0.06	0.06	0.06	0.007	0.41
Capric, C10:0	<0.01	<0.01	<0.01	<0.01	<0.01	0.001	0.80
Lauric, C12:0	0.02	0.02	0.02	0.02	0.02	0.004	0.74
Myristic, C14:0	0.04	0.04	0.04	0.04	0.04	0.004	0.67
Palmitic, C16:0	0.53	0.39	0.52	0.41	0.42	0.055	0.25
Stearic, C18:0	0.29^{a}	0.22^{ab}	0.25^{ab}	0.19^{b}	0.23^{ab}	0.022	0.04
Arachidic, C20:0	0.01	0.01	<0.01	0.01	<0.01	0.002	0.12
Behenic, C22:0	<0.01	<0.01	<0.01	<0.01	<0.01	0.001	0.94
Lignoceric, C24:0	0.01^{ab}	0.01^{a}	0.02^{ab}	0.02^{ab}	0.02^{b}	0.002	0.02
Monounsaturated							
Myristoleic, C14:1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.001	0.97
Palmitoleic, C16:1	0.11	0.09	0.10	0.09	0.10	0.011	0.81
Oleic, C18:1 *cis*	0.53	0.31	0.43	0.33	0.40	0.058	0.07
Vaccenic, C18:1 *trans*	0.15^{a}	0.11^{ab}	0.11^{ab}	0.09^{b}	0.10^{ab}	0.012	0.02
Eicosanoic, C20:1	0.01	0.01	<0.01	0.01	<0.01	0.003	0.17
Erucic, C22:1	ND[2]	ND	ND	ND	ND	-	-
Nervonic, C24:1	ND	ND	ND	ND	ND	-	-
Polyunsaturated							
Linoleic, C18:2 n-6	0.11	0.10	0.20	0.09	0.10	0.039	0.23
γ-Linolenic, C18:3 n-6	0.01	0.01	0.01	0.01	0.01	0.003	0.61
α-Linolenic, C18:3 n-3	0.01	0.01	0.02	0.01	0.01	0.006	0.51
Eicosadienoic, C20:2 n-6	0.03^{a}	0.02^{b}	0.02^{b}	0.02^{b}	0.02^{b}	0.002	<0.01
Eicosatrienoic, C20:3 n-3	<0.01^{a}	<0.01^{a}	<0.01^{ab}	<0.01^{b}	<0.01^{a}	0.001	<0.01
Arachidonic, C20:4 n-6	0.24^{a}	0.23^{a}	0.21^{a}	0.13^{b}	0.12^{b}	0.021	<0.01
Eicosapentaenoic, C20:5 n-3	0.01^{a}	0.01^{a}	0.02^{a}	0.04^{b}	0.06^{c}	0.003	<0.01
Docosahexaenoic, C22:6 n-3	0.15	0.08	0.11	0.12	0.16	0.022	0.08
Total n-3	0.17^{abc}	0.10^{a}	0.15^{ab}	0.17^{abc}	0.23^{c}	0.029	<0.01
Total n-6	0.39	0.36	0.44	0.25	0.25	0.049	0.33
n-6:n-3 ratio	2.3:1	3.6:1	2.9:1	1.5:1	1.1:1	-	-

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]Serum was collected from 12 piglets per diet (from 12 different sows per diet) at farrowing

[2]ND = not detected, limit of detection was 0.005 mg FA/ml plasma

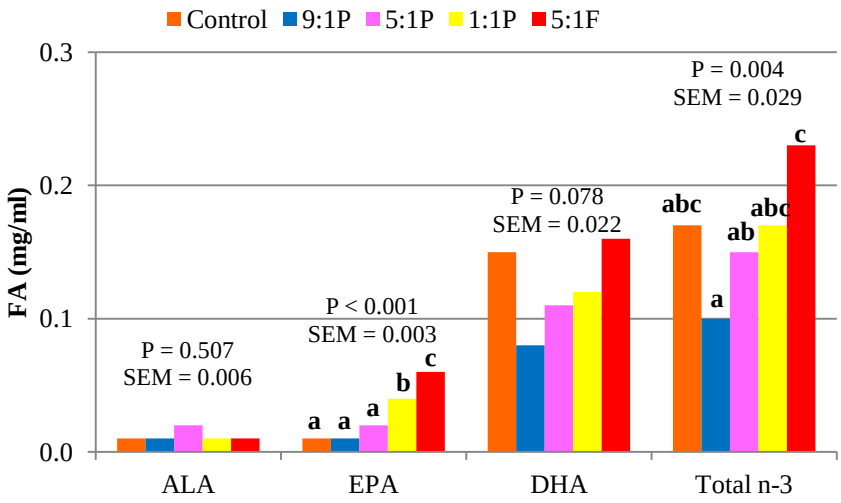

Figure 4.4: Pre-suckle piglet serum α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and total n-3 profiles collected at farrowing (n=12/diet). Treatments refer to the tallow based control and the n6:n3 ratio. Bars within each FA group without common superscripts differ ($P \leq 0.05$).

Table 4.11: Fatty acid profile of post-suckle piglet serum collected 24 hours post-farrowing[1]

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
Fatty Acid, mg FA/ml Serum	**Control**	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Saturated							
Caprylic, C8:0	0.05	0.05	0.06	0.05	0.06	0.056	0.59
Capric, C10:0	<0.01	<0.01	<0.01	<0.01	<0.01	0.004	0.78
Lauric, C12:0	0.02	0.01	0.02	0.02	0.02	0.003	0.79
Myristic, C14:0	0.06	0.07	0.07	0.05	0.07	0.011	0.45
Palmitic, C16:0	0.90	1.28	1.17	0.78	0.90	0.156	0.14
Stearic, C18:0	0.42	0.55	0.52	0.42	0.43	0.054	0.30
Arachidic, C20:0	0.01	0.01	0.01	0.01	0.01	0.001	0.66
Behenic, C22:0	<0.01^{a}	<0.01^{a}	<0.01^{a}	0.01^{b}	0.01^{b}	0.003	0.04
Lignoceric, C24:0	0.02^{a}	0.03^{a}	0.04^{ab}	0.03^{a}	0.05^{b}	0.006	0.04
Monounsaturated							
Myristoleic, C14:1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.001	0.30
Palmitoleic, C16:1	0.13	0.16	0.15	0.11	0.16	0.021	0.32
Oleic, C18:1 *cis*	0.91	1.52	1.19	0.74	0.87	0.239	0.17
Vaccenic, C18:1 *trans*	0.13	0.19	0.16	0.12	0.16	0.022	0.26
Eicosanoic, C20:1	0.01^{a}	0.02^{ab}	0.01^{a}	0.01^{a}	0.03^{b}	0.004	<0.01
Erucic, C22:1	<0.01^{a}	<0.01^{ab}	<0.01^{a}	<0.01^{a}	<0.01^{b}	<0.001	<0.01
Nervonic, C24:1	0.09^{a}	0.07^{a}	0.10^{a}	0.09^{a}	0.18^{b}	0.014	<0.01
Polyunsaturated							
Linoleic, C18:2 n-6	0.57^{a}	1.36^{b}	1.24^{b}	0.61^{a}	0.53^{a}	0.204	<0.01
γ-Linolenic, C18:3 n-6	0.01^{a}	0.02^{b}	0.02^{b}	0.01^{a}	0.01^{a}	0.002	0.02
α-Linolenic, C18:3 n-3	0.03^{a}	0.11^{ab}	0.16^{bc}	0.22^{c}	0.04^{a}	0.037	<0.01
Eicosadienoic, C20:2 n-6	0.03^{ab}	0.04^{a}	0.04^{a}	0.02^{b}	0.02^{b}	0.004	<0.01
Eicosatrienoic, C20:3 n-3	<0.01^{a}	0.01^{ab}	0.01^{b}	0.02^{b}	0.01^{ab}	0.002	<0.01
Arachidonic, C20:4 n-6	0.20^{bc}	0.30^{a}	0.27^{ab}	0.14^{c}	0.15^{c}	0.023	<0.01
Eicosapentaenoic, C20:5 n-3	0.01^{a}	0.02^{a}	0.03^{a}	0.05^{a}	0.16^{b}	0.011	<0.01
Docosahexaenoic, C22:6 n-3	0.09^{a}	0.07^{a}	0.10^{a}	0.09^{a}	0.18^{b}	0.014	<0.01
Total n-3	0.13^{a}	0.20^{ab}	0.30^{bc}	0.38^{c}	0.39^{bc}	0.088	<0.03
Total n-6	0.81^{a}	1.72^{b}	1.57^{b}	0.78^{a}	0.71^{a}	0.207	0.01
n-6:n-3 ratio	6.2:1	8.6:1	5.2:1	2.1:1	1.8:1	-	-

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]Serum was collected from 12 piglets per diet (from 12 different sows per diet) 24 hours post-farrowing. Piglets were from the same litters as the piglets used for pre-suckle sample collection

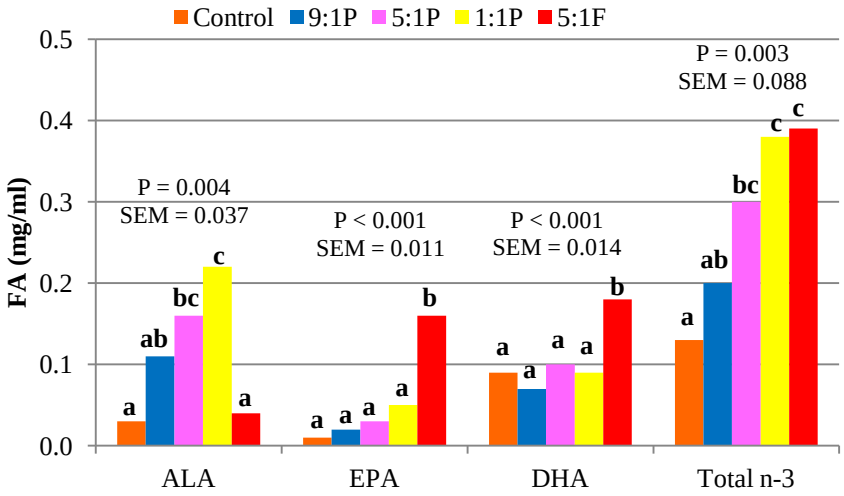

Figure 4.5: Post-suckle piglet serum α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and total n-3 profiles collected 24 hours after farrowing (n=12/diet). Treatments refer to the tallow based control and the n6:n3 ratio. Bars within each fatty acid group without common superscripts differ ($P \leq 0.05$).

Table 4.12: Serum long chain fatty acid to dietary ALA (sLC:ALAin) and serum EPA to dietary ALA (sEPA:ALAin) ratios in sows and their offspring (all values x 10^{-6}).

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F[5]**	**SEM**	***P* Value**
sLC:ALAin[1,2,3]							
Sows	13.0[a]	8.8[a]	5.6[a]	4.3[a]	75.0[b]	4.3	<0.01
Pre-suckle piglets	40.0[a]	15.0[b]	9.9[b]	5.1[b]	36.0[a]	4.9	<0.01
Post-suckle piglets	0.09	0.03	0.03	0.16	0.49	0.0	0.13
sEPA:ALAin[1,3,4]							
Sows	4.7[a]	2.8[a]	2.3[a]	1.9[a]	43.0 [b]	2.2	<0.01
Pre-suckle piglets	3.5[a]	1.9[ab]	1.2[b]	1.1[b]	9.2[c]	0.0	<0.01
Post-suckle piglets	0.001	0.001	0.001	0.005	0.024	0.0	0.07

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]A larger ratio indicates greater conversion of α-linolenic acid (ALA) into its long chain derivatives

[2]sLC is the total serum long chain fatty acids, and is the sum of circulating eicosatrienoic acid (ETA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

[3]ALAin is the dietary intake of ALA

[4]sEPA is the serum EPA content

[5]5:1F diet sows had significantly greater quantities of LC and EPA intakes, which is not represented within these ratios. Conversion of ALA into LC and EPA appears greatest for this diet; however intake amounts were greater.

4.5 Discussion

This study was designed to determine the effects of reducing the n-6 to n-3 FA ratio in sow diets on performance and FA profiles, and allowing for an estimate of potential differences in transfer efficiency and conversion to longer chain derivatives. In the literature are papers examining the responses to inclusion of n-3 FA's in sow diets, however, the treatments typically add a majority of fish oil into the diet (Leonard et al., 2010a; Rooke et al., 2000; Rooke et al., 2001a; Webel et al., 2003). In many cases however, the control diets are extremely high in n-6 FA's as they are corn based, and thus the potential benefits of n-3 inclusion may be partly masked by the high n-6 content. The ratio and the amount are confounded, and the high n-6 may mask the presence of n-3.

Within the body, there is competition between the 18 carbon n-6 and n-3 FA's for the enzymes required for elongation (Palmquist, 2009). These enzymes have a greater affinity for the n-3 FA's relative to the n-6 FA's, but due to the fact that intake of n-6 FA's are 10 to 25 times greater than n-3 FA's, there is increased conversion of LA (n-6) into arachidonic acid (ArA; n-6) when compared with the conversion of ALA into EPA (Palmquist, 2009). Typical corn based gestation and lactation sow diets contain a n-6:n-3 ratio of approximately 20:1, and this may conceal some of the potential effects of including additional n-3 FA's into sow diets. This study was designed to test the effects of reducing this dietary ratio, by reducing the level of n-6 FA's in the diets and by increasing n-3 FA's.

The diets for this study were formulated to contain equal amounts of crude fat, with only the FA ratio changing. For the control diet, a FA ratio of 8:1 existed; however, this diet contained approximately half of the total PUFA compared to the test diets. Following FA analysis of the diets, it was determined that although formulated to be a 1:1 ratio in the fish based diet, the ratio was actually 5:1, indicating that the fish oil source used (herring oil) had lower levels of EPA and DHA than previously reported in the literature and much greater quantities of ArA (NRC, 1998). Similarly, the measured ratio of n-6:n-3 for the 10:1P diet was actually 9:1 in gestation and 7:1 in lactation. The fish oil diet was included to compare the effects of plant based vs. fish based sources of n-3's at the same ratio.

Litter size and weight (number of piglets born and total litter birth weights) were similar across treatments. Average piglet birth weights were highest for those born to sows consuming the control and 5:1P diets, and were lowest for those born to sows consuming the 5:1 fish diet. The same pattern was seen with piglet average daily gain (ADG) throughout lactation, which resulted in piglets born to sows consuming the 5:1F diet having the lowest weaning weights across treatment groups.

In several studies examining dietary inclusion of n-3 FA's there have been conflicting results on reproductive performance. Rooke et al. (2000) found no effects of including linseed oil or tuna oil (17.5 g oil/kg diet total) into sow diets on live weight of piglets throughout a 28 day lactation period. A separate study however, found increases in postnatal growth with feeding 17.5 g fish oil/kg diet to sows (Rooke et al., 2001b). Additionally, they found that piglets born to sows fed fish oil had decreased birth weights, but also decreased pre-weaning mortality, while having no effect on overall litter size (Rooke et al., 2001a). Leonard et al. (2010a) reported no effects of fish oil inclusion (100 g/d) on litter size, litter weight, piglet birth weight, or piglet ADG throughout lactation. Mateo et al. (2009) also showed no differences in litter size at farrowing in gilts fed an n-3 enriched diet. Conversely, Spencer et al. (2004) found increases in litter size with reductions in piglet birth weight, and thus they observed no major effects on litter weight and Webel et al. (2003; 2004) observed increases in subsequent litter sizes after sows were fed a protected fish oil source during lactation and breeding. Similarly, Smits et al. (2011) also reported increased subsequent litter sizes when sows were fed a fish oil diet throughout lactation and breeding but not during gestation, but found no effects of the fish oil on performance during the lactation period when the diets were fed.

In the study by Rooke et al. (2000), where no effects of including flax or tuna oils were observed, the control diet was corn based and thus contained high levels of LA, an n-6 FA. Likewise, a corn based control diet was used when a protected long chain n-3 FA source was included as a top dress throughout lactation (Webel et al., 2004; Webel et al., 2003). A few studies have used a wheat and barley based control diet, similar to that used in the present study (Rooke et al., 2001a; Smits et al., 2011). These studies showed some positive effects on reproductive performance when fish based FA's were included into

sow diets, which may have become more evident due to the fact that the n-6:n-3 ratio would be much lower than those studied which used a corn based control diet.

Sow weights and weight changes throughout lactation were not different across treatment groups; however, there were differences in backfat thickness as lactation progressed. During gestation and the onset of farrowing there were no dietary effects on backfat thickness, probably because feed intakes were adjusted for body condition, but by d 7 post farrowing and again at weaning, sows consuming the lower ratio plant based diets (5:1P and 1:1P) had greater backfat thickness relative to those consuming the control diet, 9:1P and 5:1F diets. Additionally, feed intake was lowest for sows consuming the fish based diet, which may have accounted for their reduced backfat thickness.

During P1, there was a tendency for piglets born to sows consuming the 1:1P and 5:1F diets to have reduced birth weights, a pattern which continued into P2, where piglets born to sows consuming the 5:1F diet were lightest, those from the 1:1P diet were intermediate and those from the control and 5:1P diets were heaviest. The ADG for piglets raised by sows consuming the 5:1F diet was also reduced. It is possible that due to reduced feed intakes, these sows may not have been able to provide as much milk for their offspring as sows consuming the other diets.

The reason for reduced ADFI throughout lactation in the 5:1F sows is unclear. Throughout gestation and lactation for each period of the study, there were no sows that refused to consume their feed. Palatability or taste of the fish based diet may be a factor. In the present study, fish oil was included at 38 g/kg diet as fed. This value is slightly higher than the average found in the literature. Rooke et al. (1998); 1999) used an inclusion rate of 30 g/kg diet; Rooke et al. (2001a) and Smits et al. (2011) used 33 g/kg and Rooke et al. (2000) used 35 g/kg of fish oil.

All oils were stored with antioxidants included and ethoxyquin was added to each diet to ensure that rancidity did not become an issue when including high levels of PUFA’s. Diets were also made in small batches on a frequent basis (approximately every 2 to 3 months) to ensure that no diet was stored in bins for extended periods of time. Upon chemical analysis, the fish oil diet contained greater quantities of total fat relative

to the other diets (55 vs. 45-50 g/kg). It is possible that since energy density of this diet would have been slightly greater, the sows reduced their feed intake to compensate.

There was an increase in the number of stillbirths and mummified piglets as the n-6:n-3 FA ratio in sow diets decreased (Figure 4.1 Figure 4.2) relative to the control diet. In the few studies reporting effects of dietary n-3 FA's on mummified or stillborn piglets, there was no evidence that including fish oil increased the number of piglets born dead (Mateo et al., 2009; Smits et al., 2011). One possible explanation for the increase in piglets born dead as the n-6:n-3 ratio decreased is reduced prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is the leuteolytic hormone that initiates farrowing. Nara and First (1980) showed that in order to have a normal and rapid delivery, high levels of $PGF_{2\alpha}$ are required. Perhaps the reduction in dietary n-6 FA's led to a decrease in $PGF_{2\alpha}$ and thus an increase in the length of time required for farrowing. This may then have led to increases in the number of piglets born dead. Unfortunately, we do not have data on the dietary effects on the length of farrowing period. Fraser et al. (1997) has shown that increased time for farrowing and/or increased time between births is correlated with increased stillbirths.

Production data for this experiment was initially analyzed as a randomized complete block to account for parity, but after determining that the effect of parity was not significant for any parameter measured it was removed from the model. The statistical model did not include all possible sources of variation such as the effects of litter size or backfat change in the sows, because our objective was to examine the effects of diet over time on lactation performance. If we included covariates such as litter size in the model, the model would no longer account for dietary effects throughout the breeding and gestation period. Similarly, the inclusion of backfat thickness at farrowing as a covariate in the model would not allow for determination of the effects of treatment on backfat changes over time.

There have been several studies in the literature that have looked at the effects of dietary FA profiles on immunoglobulin content in sow colostrum. Although the mechanisms relating n-3 FA's to immunoglobulins are not well understood, it appears that IgA and IgG may be affected by the presence of n-3 FA's. Mateo et al. (2009) observed that feeding fish based n-3 FA's increased the IgG content of colostrum by 5 to 10 mg/mL. In a study with human breast milk, Dunstan et al. (2004) found that IgA

content was positively correlated with DHA intake, and negatively correlated with LA intake, and thus intake of n-3 FA's in the form of fish oil could improve IgA status of the offspring. In a study in which sows were fed high levels of corn oil and thus high levels of the n-6 FA LA, reductions in colostral IgG content were observed (Jackson et al., 1995). We found no effect of altering the dietary n-6:n-3 FA ratio on IgG or IgA concentrations in colostrum or piglet serum, similar to the findings of Leonard et al. (2010a), who found no changes in IgA or IgG amounts in the colostrum and milk of sows consuming additional fish oil, or in the plasma of their offspring. In previous studies, total fat content of the diets was not reported as being held constant across treatment groups, and thus added fat to the diet may have increased the total fat content of milk or colostrum (Pettigrew, 1981). In this experiment, the total fat content of diets maintained relatively consistent with only the specific FA's changing, and thus may be the reason that no effects of diet were observed in terms of the immunoglobulin content.

Although there were alterations in the amounts of the FA's provided to the piglets by the colostrum and milk, the overall profile remained similar, with the n-6:n-3 ratio's closely mimicking those of the sow diets. Because of this, we can conclude that feeding specific n-6 and n-3 FA profiles to sows will transfer to their offspring via milk. This is similar to the findings of many studies in which the colostrum and milk FA profile mimics that of the sow's diet when fish oils were fed (Fritsche et al., 1993; Lauridsen and Danielsen, 2004; Leonard et al., 2010a; Mateo et al., 2009; Rooke et al., 1998; Rooke et al., 2000; Rooke et al., 2001a) or when flax oil was fed (Farmer and Petit, 2009), and is characteristic of monogastric animals. Similarly, the profile of FA's in sow serum and in the serum of their offspring was consistent with that found in the diet and milk.

The FA profile of pre-suckle piglet serum indicates that the conversion of ALA into EPA can be increased by reducing the dietary n-6:n-3 FA ratio of their sows. It is possible that reduced competition for the enzymes responsible for the desaturation (Δ5- and Δ6-desaturases) and elongation (elongase) occurred, allowing for increased selection of the n-3 FA's by these enzymes thus improving conversion efficiency. In a study by Missotten et al. (2009), Δ5- and Δ6-desaturase enzyme expression was upregulated in a tissue specific manner in the offspring of sows consuming a fish or flax oil diet. They found that fish oil in the diet increased the expression of Δ5- and Δ6-desaturase enzymes

in muscle tissue. With increased n-3 FA's in the diet and thus increased n-3 substrate availability, reductions in competition between n-6 and n-3 FA's for the desaturase and elongase enzymes may have occurred. This, combined with possible increased expression of the desaturase enzymes, may have led to the increased conversion of ALA into EPA seen in the present study.

After consumption of colostrum, the serum FA ratio in piglets more closely mimicked that of the sow diet and colostrum profile; however, the circulating EPA concentration post-suckle was similar to that of pre-suckle piglet serum. Changes to body FA composition observed from reductions in the dietary n-6:n-3 FA ratio can be seen post-suckling; however, it appears that the majority of EPA present in piglets was from placental transfer or fetal conversion in utero.

When examining the serum long chain FA to dietary ALA ratios, it appears that the diet eliciting the greatest conversion efficiency was the 5:1F diet. However, this would appear to be an artifact of the dietary EPA and LC intake levels. The ratio calculation is dietary ALA intake to serum EPA or LC levels, and does not account for the possibility of dietary EPA intake. The 5:1F diet, contained EPA, which then can be found in the serum, and is unaccounted for in the calculation. In order to account for the dietary EPA or LC intake, details on the specific bodily uses of each individual FA would be essential, which would require use of an isotope labelling experiment. The dietary EPA intake was similar across all plant based diets, and thus differences in dietary intake would not be of major concern in the calculation of these ratios. Conversely, the fish based diet had significantly more EPA and LC amounts, and would affect the ratio, thus caution should be used when interpreting the conversion ratio for that treatment. The sows consuming the 5:1F diet had significantly higher intakes of the long chain n-3 FA's compared with all other diets, and thus had a greater transfer of these FA's into serum. As discussed by Welch et al. (2010), a higher ratio indicates an improved conversion efficiency of ALA into EPA or total long chain n-3's. In the current experiment, this ratio may not explain the full effects of diet on conversion efficiency because it does not account for n-6 or long chain n-3 intakes. When looking at piglet serum FA's, we can clearly see that as the n-6:n-3 ratio decreased in sow diets, piglets had elevated amounts of circulating EPA, and thus increased conversion of ALA into its longer chain

counterpart. In order to get a proper estimate of this conversion efficiency, tracing specific dietary FA's and conversions would be required, (i.e. using isotope labelling) which was not included in the current study.

4.6 Conclusions

Results from this study indicate that the reproductive performance of sows fed diets with decreasing plant based n-6:n-3 ratios were similar to those consuming the control diet which was high in saturated FA. Piglets raised by sows consuming the plant based diets had similar birth and weaning weights when compared to the control diet, and litter size was not altered. When fed a fish based 5:1 diet, sow feed intakes were reduced and, piglets were born and weaned at lighter weights relative to the other dietary groups. Stillbirths increased in all treatment groups relative to the control diet, and there was a tendency for increased stillbirths as the n-6:n-3 ratio decreased.

This study has shown that feeding diets with decreased n-6:n-3 ratios to sows alters the circulating FA profile in sows and their offspring. Offspring of sows fed a plant based 1:1 n-6:n-3 ratio had increased circulating EPA relative to the control, 10:1P and 5:1P groups, indicating that plant based n-3 sources may be an alternative to feeding fish oils to pigs. In order to obtain the increased circulating EPA levels from feeding plant based n-3 FA's, it is important to account for the n-3 content relative to n-6 FA's.

5 EFFECTS OF ALTERING THE OMEGA-6 TO OMEGA-3 FATTY ACID RATIO IN SOW DIETS ON BODY FAT MOBILIZATION DURING LACTATION IN HIGH PRODUCING SOWS

5.1 Abstract

An experiment was designed to test the hypothesis that reducing the sow dietary omega-6 (n-6) to omega-3 (n-3) fatty acid (FA) ratios would increase body fat mobilization and nutrient output in the milk, thus improving reproductive performance, which we characterized using weaning rates, piglet survival and growth performance. The objectives of the trial were to determine if changing the sows' FA profile intake would affect sow body condition by altering body fat mobilization during lactation, and if milk production and piglet growth performance would be altered.

Sows were assigned to one of five diets on d 80 of gestation and remained on these diets for 3 reproductive cycles; data was collected for this experiment during the third cycle (Period 3; P3). Wheat and barley based diets were divided into gestation and lactation rations. A constant total fat level was maintained (5% crude fat) with only the amount and ratio of polyunsaturated fatty acids (PUFA) changing between diets. The treatments consisted of a control diet (tallow based), 3 diets with plant oil based n-6:n-3 ratios (9:1P, 5:1P, and 1:1P) and a 5:1 fish oil diet (5:1F). The control diet had a ratio of 8:1 but contained approximately half the total PUFA compared to the other diets. Sows were assigned to treatment if they farrowed ≥ 11 piglets and were required to nurse ≥ 10 piglets; a total of 20 sows per diet were used. Milk samples were collected on d 4 and d 16 of lactation. Piglet ADG and sow feed intake were determined. Dry matter (DM), N and energy output of milk were estimated based on piglet growth rate. Jugular catheters were inserted into 8 sows from each of the 9:1P and 1:1P groups on d 5 of lactation and sows were given a single injection of epinephrine followed by serial blood collections for 2 h to determine dietary effects on the sows' ability to mobilize body fat. Plasma leptin was determined on d5 and 15 of lactation.

Throughout the whole lactation period, feed intake was greatest for sows consuming the control (8.4 kg/d) and 5:1P (8.2 kg/d) diets, lowest for sows fed the 1:1P (7.4 kg/d) diet and intermediate for the 9:1P (7.7 kg/d) and 5:1F (7.7 kg/d) diets ($P = 0.05$). Altering the n-6:n-3 FA ratio did not affect sow BW, piglet ADG or milk DM, N or total output ($P > 0.2$). Sows

consuming the 1:1P diet had greater backfat thickness ($P < 0.05$) and numerically higher plasma NEFA at baseline (d 5) when compared to the 9:1P sows (240 vs 93 uM; $P = 0.16$). When challenged with epinephrine, sows fed the 9:1P diet tended to have a lower net incremental area under the curve (niAUC) for glucose ($P = 0.08$) and numerically higher, but nonsignificant niAUC NEFA ($P = 0.17$) and glycerol ($P = 0.15$) relative to the 1:1P sows. Additionally, sows with greater backfat thickness (1:1P group) increased circulating leptin concentrations and reduced feed intakes, which were correlated with increased body fat mobilization (increased plasma NEFA and glycerol)

Relative to sows fed the 9:1P diet, sows fed a plant based dietary n-6:n-3 FA ratio of 1:1 appeared to be in a state of negative energy balance, evidenced by increased body fat mobilization prior to epinephrine stimulation. However, as no dietary effects were observed on piglet growth rates, it appears that these sows were able to provide the same level of nutrients to their offspring as the 9:1P fed sows.

Keywords: fat mobilization, lactation, non-esterified fatty acids, omega-3, omega-6, sow

5.2 Introduction

Improved genetics and management practices have led to increased litter sizes for sows; however, pre-weaning mortality rates have also increased (Edwards and Baxter, 2012), partly because sows may not be able to provide enough milk to meet the demands of these larger litters. As piglet numbers increase, there is an increasing demand on the sow to provide milk. At the time of farrowing, sows undergo many metabolic changes associated with milk production which can put them into negative energy balance (Pettigrew et al., 1993). If the sow cannot consume enough feed to meet the energy demands of milk production, she will often draw on her own body fat reserves. Hypophagia at farrowing contributes to the sow's inability to meet her energy demands for milk production, and over subsequent parities, severe negative energy balance and the loss of body condition can have negative impacts on subsequent rebreeding performance and may lead to early culling from the herd (Clowes et al., 1998; Noblet et al., 1990).

Altering the fatty acids (FA) in adipose tissue can affect lipolytic activity and the ability of the animal to mobilize body fat (Tilton et al., 1999). Omega-3 (n-3) FA's perturb lipid metabolism (Chan et al., 2003; Harris, 1997; Lee et al., 2008), and may also affect feed intake. Moreover, it is possible that the ratio of n-3 FA's in relation to omega-6 (n-6) FA's will differentially affect body fat mobilization in the sow (Papadopoulos et al., 2009a). It is well known that the FA profile of adipocytes reflects the composition of dietary lipids (Eastwood et al., 2009; Fickova et al., 1998); however, the effect of n-3 FA's on lipogenesis and lipolysis is less clear.

The objective of this trial was to determine if altering the n-6:n-3 FA ratio in sow diets throughout gestation and lactation would affect body condition (back fat), milk production and piglet performance throughout lactation. It is hypothesized that high producing sows (≥ 11 piglets farrowed, ≥ 10 piglets nursed) consuming diets with reduced n -6:n-3 FA ratios have increased body fat mobilization and increased nutrient output in their milk. Specifically, we aimed to determine how altering the dietary n-6:n-3 FA profile would affect whole body metabolism and the sows ability to provide nutrients and energy to her offspring by measuring piglet growth rate (and estimating milk energy output), sow feed intake, and the lipolytic activity of the adipose tissue following stimulation.

5.3 Materials and Methods

The housing, diets and animal management were described in Chapter 4. Experimental procedures, sample collections and data analysis differed.

5.3.1 General

This experiment was approved by the University of Saskatchewan's Animal Research Ethics Board (UCACS #'s 19970020 and 20090129), and adhered to the Canadian Council on Animal Care guidelines for humane animal use (CCAC, 1993). The experiment was conducted at the Prairie Swine Centre Inc. (Saskatoon, Saskatchewan, Canada), a 300 sow farrow to finish facility. Sows and piglets were housed in temperature-controlled rooms according to the thermoneutral zone for the specific age and stage of reproduction (Zhang, 1994). Additional heating was provided to nursing piglets in the form of heat lamps within the farrowing crate. Lighting was maintained on a 12 h light:dark cycle (07:00 – 19:00) throughout the course of the experiment. All pigs were PIC genetic lines (Camborough Plus females x C3378 sires, PIC Canada Ltd., Winnipeg, Manitoba, Canada). Flaxseed meal (FSM) and flaxseed oil (FSO) were obtained from Vandeputte S.A (Mouscron, Belgium), and the chemical composition of this FSM product has been previously described by Eastwood et al. (2009).

5.3.2 Animals and Housing

This experiment utilized 100 sows (240 ± 33 kg, parity 2 to 5; n=20/treatment) in a completely randomized design. Sows were selected from a larger group of 150 which were utilized in the experiment described in Chapter 4. Each sow was randomized to one of five treatment groups based upon their expected farrowing dates, and treatments were balanced across parities. Start dates for sows were staggered within the trial, as only 8 to 12 sows were available each week. To meet our objectives, sows were required to farrow ≥ 11 piglets and were required to nurse≥ 10 piglets, and had been on their experimental diets for approximately 7 months prior to be included in this trial. All animals were managed according to standard production practices throughout breeding, gestation and farrowing, and all piglets were cared for

under normal operating procedures. Cross-fostering of piglets occurred within the first 24 hours of farrowing when required, but could not always occur within treatment groups.

Throughout gestation all animals were housed in a free access stall system as described in Chapter 4. On d 110 of gestation sows were moved from the gestation facility to a farrowing room equipped with 16 individual farrowing crates. Sows were housed in the crates for a lactation of approximately 4 weeks. During the final week of lactation, creep feed was provided to piglets.

5.3.3 Treatments and Feeding

A total of 5 dietary treatments were used for this experiment, each divided into a gestation and a lactation ration (10 diets). These diets are identical to those described in Chapter 4 and shown in Table 4.1a/b and Table 4.2a/b. Sows consumed these diets for approximately 7 months prior to this section of the trial.

Diets were balanced for net energy content and digestible essential amino acids as recommended for gestating and lactating sows (NRC, 1998). Diets were wheat and barley based, and formulated based on digestible oil content to contain equal amounts of crude fat (5%). The diets were prepared frequently (approximately every 3 months as required) and fed in pellet form. Oils with different polyunsaturated FA profiles were supplemented into the diets to adjust the ratio of n-3 to n-6 FA's while maintaining constant total fat levels. The treatments consisted of a control (tallow based, low in PUFA), 3 diets with plant oil based n-6:n-3 ratios (9:1P, 5:1P, and 1:1P) as well as a 5:1 fish oil diet (5:1F). The 5:1F diet was originally formulated as a 1:1 ratio; however FA analysis revealed this was not the case. Similarly, the diet formulated to contain a 10:1 ratio was analyzed to contain a 9:1 ratio in gestation and 7:1 in lactation. Ethoxyquin was added (0.025% inclusion) to all diets to reduce the risk of FA oxidation and potential problems with diet rancidity.

Sows were fed 2.5 to 3.0 kg of feed per day (at 08:00) throughout breeding and gestation dependent upon their body condition. Two weeks prior to farrowing their feed intake was increased to 3.0 to 3.5 kg per day. Post-farrowing, sows were fed *ad libitum* as appetite increased. Water was provided *ad libitum* throughout the trial.

5.3.4 Experimental Procedure

Sows began their assigned diets approximately 7 months prior to the start of this breeding to weaning cycle, as described in Chapter 4. A group of 100 high-producing sows were selected from the overall group of 150 sows. In order to be classed as a high-producing sow she was required to farrow≥ 11 piglets and to nurse ≥ 10 piglets. A total of 20 sows per diet were used. Production data was collected for each of these sows. The two reproductive cycles described in Chapter 4 were referred to as Period 1 (P1) and Period 2 (P2). Period 3 (P3) refers to the high-producing sows selected to remain on trial.

During P3, sows were weighed on d 110 of gestation, within 24 hours post farrowing, d 7 post farrowing and weaning. Backfat thickness was determined using a Real Time (B Mode) Ultrasound Scanner (Pie Scanner 200 SLC, Pie Medical, The Netherlands) when the sows were weighed. The right side of each animal was scanned longitudinally between the 10^{th} and last ribs, 5 cm lateral to the dorsal midline. To ensure that scan location was identical for each measurement, the location was marked on each sow during the first scan.

The total number of piglets born, as well as mummies and stillbirths were recorded. Piglets were weighed immediately following farrowing, and on d 3, 7, 10, 14, 21 and 28 of lactation. Milk production and energy output in the milk was calculated from piglet growth rate, up to d 21 post-farrowing, according to the equations of Noblet and Etienne (1989). For all estimation equations, they report a minimum R^2 of 0.81 between sow milk DM, and energy and nitrogen in milk from litter BW gain. This method was chosen over the weigh-suckle-weigh method of measuring milk output or isotope dilution because it does not disrupt the normal social interaction of the sow and litter (Pettigrew et al., 1985).

The Noblet and Etienne (1989) equations used to calculate milk production (M), as well as dry matter (DM_L), energy (E_L) and nitrogen (N_L) for d 1 to 21 of lactation are as follows:

$$M = 2.50\ (\pm\ 0.26) \times ADG + 80.2\ (\pm\ 7.8) \times BW_i + 7 \quad \textbf{(5.1)}$$

$$DM_L = 0.401\ (\pm\ 0.044) \times ADG + 12.9\ (\pm\ 1.3) \times BW_i + 19 \quad \textbf{(5.2)}$$

$$E_L = 2.54\ (\pm\ 0.34) \times ADG + 78.7\ (\pm\ 10.2) \times BW_i + 153 \quad \textbf{(5.3)}$$

$$N_L = 0.013\ (\pm\ 0.002) \times ADG + 0.372\ (\pm\ 0.057) \times BW_i + 1.86 \quad \textbf{(5.4)}$$

where ADG is the piglets average daily gain throughout the 21 day period (g) and BW_i is the body weight of the piglet at birth (kg); M, DM_L and N_L are in g per piglet per day, and E_L is expressed as kcal per piglet per day.

Similarly, the equations used for the estimation of the d 1 to 5 period of lactation are:

$$M = 2.64\ (\pm\ 0.39) \times ADG + 67 \quad \textbf{(5.5)}$$

$$DM_L = 0.558\ (\pm\ 0.086) \times ADG + 10 \quad \textbf{(5.6)}$$

$$E_L = 3.86\ (\pm\ 0.69) \times ADG + 45 \quad \textbf{(5.7)}$$

$$N_L = 0.024\ (\pm\ 0.005) \times ADG + 0.73 \quad \textbf{(5.8)}$$

where ADG is the piglets average daily gain throughout the 5 day period (g); M, DM_L and N_L are in g per piglet per day, and E_L is expressed as kcal per piglet per day.

Estimations were also made for the period of lactation commencing on d 5 until d 21 using the following equations established by Noblet and Etienne (1989):

$$M = 1.83\ (\pm\ 0.31) \times ADG + 72.9\ (\pm\ 8.1) \times BW_i + 176 \quad \textbf{(5.9)}$$

$$DM_L = 0.296\ (\pm\ 0.053) \times ADG + 12.2\ (\pm\ 1.4) \times BW_i + 43 \quad \textbf{(5.10)}$$

$$E_L = 1.80\ (\pm\ 0.40) \times ADG + 76.4\ (\pm\ 10.3) \times BW_i + 318 \quad \textbf{(5.11)}$$

$$N_L = 0.010\ (\pm\ 0.002) \times ADG + 0.395\ (\pm\ 0.058) \times BW_i + 2.42 \quad \textbf{(5.12)}$$

where ADG is the piglets average daily gain throughout the d 5 to 21 period (g), and BW_i is the body weight of the piglet at birth (kg); M, DM_L and N_L are in g per piglet per day, and E_L is expressed as kcal per piglet per day.

A milk sample was taken from all functional teats following an intra-vulva injection of oxytocin (Oxyto-Sure 20 IU/ml, 1 ml; Vetoquinol, Lavaltrie, Quebec, Canada) on d 4 and 16 of

lactation. A total of 10 to 20 ml was collected per sow per time period. Milk was frozen at -20^{o}C until further analysis.

A subset of sows from 2 of the 5 treatments (9:1P and 1:1P) were randomly selected and used to evaluate the effect of the n-6:n-3 ratio on fatty acid mobilization from adipose tissue (n=10 for 9:1P diet; n=8 for 1:1P diet). These two diets were chosen to allow for the comparison of the two extreme n-6:n-3 ratios, with the total PUFA content held constant. The 9:1P diet was selected over the control diet, as the ratios were similar, but total PUFA was similar to the 1:1P diet. On d 5 of lactation, sows had a jugular catheter inserted into the lateral auricular vein according to the method of Niiyama et al. (1985). Catheter patency was maintained with a solution of sterile heparinized saline (0.1 % heparin).

An epinephrine challenge was conducted on d 5 of lactation according to the methods of Tilton et al. (1999) and Mersmann (1986). An epinephrine dose of 1.6 μg/kg BW was used (Tilton et al., 1999). Blood samples were collected 15 min pre-infusion and at 0, 2, 4, 6, 10, 15, 20, 30, 45, 60 and 120 minutes post-infusion. Samples were collected into evacuated blood tubes containing EDTA as an anti-coagulant. Plasma was separated by centrifugation at 830 × *g* for 15 min (Beckman TJ-6 Centrifuge, Beckman Coulter, Mississauga, Ontario, Canada) and kept frozen at -20^{o}C until analysis.

Similarly, a glucose challenge to evaluate the effect of the n-6:n-3 ratio on tissue sensitivity to insulin occurred on d 6 of lactation. Sows were infused with glucose (1 mg/kg BW) following a 12 hour fast (Tilton et al., 1999). Blood samples were collected in the same manner as those from the epinephrine challenge except additional samples were taken at 150 and 180 minutes post-infusion.

Concentrations of leptin in serum were determined on d 6 using the 15 minute pre-infusion blood sample, and on d 15 of lactation using a sample collected by jugular venipuncture into evacuated blood collection tubes with no additives. Serum was separated by centrifugation at 830 × *g* and stored at -20^{o}C until leptin analysis was conducted.

5.3.5 Analytical Methods

Proximate analysis of diets was performed by a commercial laboratory (Central Testing Laboratory Ltd (Winnipeg, Manitoba, Canada). Measures included DM (method 930.15; AOAC, 1990), ash (method 923.03; AOAC, 1990), nitrogen (Leco Analyzer, St. Joseph, MI), crude fat (ANKOM XT20), crude fibre (AOCS Ba6a-05), acid detergent fibre (ANKOM 08-16-16), lignin (ANKOM 3/98), calcium and phosphorus (methods 968.08 and 935.13A; AOAC, 1990).

Diet FA profile was determined using GLC (Agilent 6890 with Agilent ChemStation Software; Agilent Technologies, Mississauga, Ontario, Canada). Direct FA methylation was performed according to the procedure of O'Fallon et al. (2007). Non-methylated C13:0 (Nu-Chek Prep Inc, Elysian, MN) was used as the internal standard, and all other chemicals were GLC grade (Sigma-Aldrich Inc., St. Louis, MO). Fatty acid methyl ester (FAME) samples were compared with a standard mixture containing a wide array of FAME's ranging from C8:0 to C24:1 (GLC-68-D, GLC-97 and U-62-M; Nu-Chek Prep Inc, Elysian, MN) using a GLC program slightly modified from the procedure described by O'Fallon et al. (2007). Briefly, the machine was set for a 1.0 μl injection and split at a ratio of 30:1. The injector set points were a temperature of 260^{o}C, pressure of 40.24 psi, and a total flow for the carrier gas (helium) of 37.5 mL/min. The initial oven temperature of 140^{o}C was held for 5 min. The temperature was ramped up at a rate of 4^{o}C/min to a maximum of 240^{o}C and held for 15 min. The total run time was 45 min. A Supelco fused silica capillary column SP 2560 (Sigma-Aldrich Inc., St. Louis, MO) was used. A flame ionization detector was utilized for detection, with the heater set at 250^{o}C, hydrogen flow of 40 mL/min, air flow of 450 mL/min and helium flow of 45 mL/min.

Epinephrine challenge samples were analyzed for glucose, NEFA and glycerol using colorimetric test kits purchased from BioAssay Systems (Hayward, CA; catalogue numbers DIGL-200, EFFA-100 and EGLY-200 respectively). The glycerol assay had a linear sensitivity between the range of 10 to 1000 μM and an intra-assay CV of 9.1%. The linear sensitivity range for NEFA was 7 to 1000 μM with an intra-assay CV of 10.6%. The glucose kit had a linear sensitivity range of 0.7 to 300 mg/dL, with an intra-assay CV of 5.9%. Glucose challenge samples were analyzed for glucose, and C-peptide (pro-insulin) using a porcine specific C-peptide RIA kit (Cat. # PCP-22K; Millipore Corp., Billerica, MA; intra-assay CV was 9.0%). Leptin was analyzed using a multi-species leptin RIA kit (Cat. # XL-85K; Millipore Corp., St.

Charles, MO; intra-assay CV was 5.6%). In order to obtain standardized results with the multispecies kit, leptin is reported as a human equivalent (HE) value. Leptin and C-peptide analyses were conducted at the Western College of Veterinary Medicine, University of Saskatchewan (Department of Veterinary Biomedical Sciences). Milk samples were analyzed for total solids (dry matter) using AOAC method 990.20 (AOAC, 1990).

5.3.6 Statistics

Data was analyzed using the Mixed Model procedure of SAS (version 9.2; SAS Inst. Inc., Cary, NC) for a completely randomized design. Performance data, milk composition data and baseline blood data were analyzed as single time points, with sow as the random effect and diet as a fixed effect. The initial statistical model included sow parity as a block (randomized complete block design); however this was non-significant and removed from the model.

For time course data (epinephrine and glucose challenges), area under the curves (AUC) were calculated using the pre infusion samples as the baseline (Tilton et al., 1999) and were analyzed using the completely randomized design in Proc Mixed. Sow was considered the random effect with AUC parameters as the fixed effects. Total AUC and net incremental AUC (niAUC) values are presented, with niAUC accounting for baseline concentrations prior to challenge.

Leptin concentrations were analyzed separately for each time point (d5 and 15) as a completely randomized design. Sow was the random effect and diet was the fixed effect.

The relationships between serum parameters in the sow and piglet growth rate (as well as other performance measures) were determined using correlation analysis using the Proc Corr procedure within SAS. For all data, significance was declared when $P \leq 0.05$. Tendencies were declared when $P < 0.1$ but > 0.05, and Tukey's honestly significant difference was used for means separation.

5.4 Results

Table 5.1 shows the estimated production of milk, as well as the DM, N and energy contents on a piglet per day basis. The calculated estimations are for d 1 to 5 and d 5 to 21 of lactation, as well as for the overall d 1 to 21 period. The measured milk DM (total solids) contents for d 4 and 16 of lactation are shown in Table 5.2. Altering the n-6:n-3 FA ratio of sow diets did not affect the overall estimated milk composition (DM, N, GE) as shown.

The effects of altering the FA ratio in sow diets on piglet performance and growth during P3 is shown in Table 5.3. There were no diet effects on piglet birth and weaning weights, and there was no effect on average daily gain of the piglets throughout lactation ($P > 0.05$).

Sow feed intake was measured as daily disappearance from farrowing to d 3 of lactation, and then total disappearance until weaning (Table 5.4). Sows consuming the control diet and 5:1P diet ate the most feed, while the 1:1P and fish diet sows consumed the least ($P = 0.05$). There was no significant effect of diet on early lactation feed intake. However, there was a high variability between animals in early lactation and a reduced number of sows during this experimental cycle when compared to P1 and P2. As the time period over which feed intake was measured increased, variability between sows decreased, and sows consuming the 1:1P diet ate significantly less feed when compared to the control and 5:1P diet sows over the whole lactation period.

Sows consuming the 9:1P and 1:1P diets underwent a metabolic challenge with exogenous epinephrine to determine the effects of reducing the n-6 to n-3 FA ratio in the diet on the maximal lipolytic activity of sow adipose tissue. The effects of diet on the baseline (pre-challenge) concentrations of plasma NEFA, glycerol and glucose are shown in Table 5.5. Circulating glucose was numerically higher in sows fed the 9:1P n-6:n-3 FA ratio relative to those fed the 1:1P ratio ($P = 0.11$). Both NEFA and glycerol concentrations were numerically greater in the 1:1P sows at baseline when compared to the 9:1P sows; however, this did not reach statistical significance.

Table 5.1: Estimated[1] milk production, dry matter, nitrogen and energy in milk

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Number of Sows[2]	12	12	13	12	11	-	-
Milk output, g/piglet/d							
d 1-21	789.3	760.3	736.1	727.0	736.2	24.84	0.38
d 1-5	534.6	500.0	533.7	488.0	479.8	25.26	0.35
d 5-21	821.1	808.8	771.0	770.5	781.2	20.24	0.25
Dry Matter, g/piglet/d							
d 1-21	144.5	139.9	136.0	134.5	136.0	3.99	0.38
d 1-5	108.8	101.5	108.6	99.0	97.2	5.34	0.35
d 5-21	148.0	154.9	139.9	139.7	141.5	3.28	0.25
Nitrogen, g/piglet/d							
d 1-21	5.9	5.7	5.6	5.5	5.6	0.13	0.38
d 1-5	5.0	4.7	5.0	4.6	4.5	0.23	0.35
d 5-21	5.9	5.9	5.7	5.7	5.7	0.11	0.25
Energy, kcal/piglet/d							
d 1-21	943.6	914.4	889.6	880.7	890.0	25.17	0.38
d 1-5	728.6	678.1	727.4	660.5	648.5	36.93	0.35
d 5-21	959.6	947.2	910.3	909.2	919.9	20.03	0.25

[1]Estimations based on equations provided by Noblet and Etienne (1989)
[2]Sows nursed 10-12 piglets on average throughout lactation

Table 5.2: Total solids (% DM) in milk samples on d 4 and 16 of lactation

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Number of Sows	13	14	14	13	11	-	-
d 4	18.57	19.12	18.67	19.43	20.29	0.553	0.23
d 16	19.42	18.45	19.50	19.30	19.01	0.353	0.23

Table 5.3: Effects of sow dietary fatty acid ratio on litter size, piglet weight and growth throughout lactation following 11 months of dietary consumption

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Number of Sows	19	19	20	18	19	-	-
Avg. Number Liveborn	12.9	12.6	12.3	12.5	13.9	0.64	0.38
Avg. Birth Wt., kg	1.41	1.35	1.40	1.31	1.26	0.057	0.30
Avg. Wean Wt., kg	8.63	8.42	8.30	7.73	8.16	0.273	0.16
ADG, kg/d	0.27	0.27	0.26	0.26	0.25	0.009	0.38

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$).

Table 5.4: Effects of dietary fatty acid ratio on lactation feed intake following 11 months of dietary consumption

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Number of Sows	19	19	20	18	19	-	-
Feed Consumed d 0-3, kg	16.3	17.8	15.3	14.6	14.1	1.65	0.46
ADFI d 0-3, kg/d	4.1	4.4	3.8	3.7	3.5	0.41	0.46
ADFI d 0-26, kg/d	8.4[a]	7.7[ab]	8.2[a]	7.4[b]	7.7[ab]	0.27	0.05

[a-b]Within a row, means without a common superscript differ ($P \leq 0.05$)

Shown in Table 5.6, the net incremental area under the curve (niAUC; AUC minus baseline) and peak concentrations for NEFA and glycerol were not different between the two dietary treatments during the epinephrine challenge period ($P > 0.10$). The niAUC was however, numerically lower for the 1:1P pigs in both cases. Again, there was high variability between animals. Sows consuming the 9:1P diet tended to have lower glucose peaks when adjusted for baseline and lower niAUC responses during the epinephrine challenge ($P = 0.09$). Results of the glucose, NEFA and glycerol responses to the challenge are shown in Table 5.6 and Figure 5.1, Figure 5.2 and Figure 5.3.

After a 12 h fast, 9:1P and 1:1P fed sows were challenged with glucose to determine the effect of diet on insulin sensitivity of sow tissues. Table 5.5 outlines the baseline concentrations of fasted glucose and C-peptide (pre-insulin). There was no effect of dietary FA ratio on the circulating concentration of either glucose or C-peptide prior to challenge ($P > 0.10$).

No effects of diet on peak glucose concentration, glucose response, peak C-peptide concentration or C-peptide response during the glucose challenge were observed ($P > 0.10$; Table 5.7). Peak glucose, regardless of dietary treatment during the fasted glucose challenge was similar to the baseline values for fed sows, indicating that a rapid increase in plasma glucose occurred during the challenge. Peak C-peptide values rose above baseline values during the challenge regardless of diet. Sows responded to the glucose challenge; however this response was unaffected by dietary treatment.

Plasma leptin concentration was determined on d 5 and 15 of lactation. As shown in Table 5.5, there was no effect of dietary treatment on the leptin concentration on d 5 ($P > 0.10$). However, sows consuming the 1:1P n-6:n-3 ratio diet tended to have elevated leptin concentrations on d 15 ($P = 0.07$).

As discussed previously, there was no difference between the 9:1P and 1:1P ratio sows in terms of total feed intake throughout lactation (Table 5.4). When looking at the early lactation feed intake of sows (d 0 to 3), sows on the 9:1P diet ate 3 kg more feed during that time than the 1:1P diet sows; however, significance was not achieved ($P = 0.46$). Correlations between leptin (d 5 or 15) and feed intake (d 0 to 3, total and ADFI) were not significant ($P > 0.10$).

Table 5.5: Baseline plasma concentrations of glucose, NEFA, glycerol, C-peptide and leptin in lactating sows fed diets containing n-6:n-3 fatty acid ratios of 9:1P or 1:1P

	Dietary Treatment (n-6:n-3 fatty acid ratio)		Statistics	
	9:1P	**1:1P**	**SEM**	**P-Value**
Number of Sows	10	8/7[1]	-	-
Fasted Glucose, mg/dL	64.67	63.54	5.701	0.88
Fed Glucose, mg/dL	78.93	56.48	9.818	0.11
Fed NEFA, uM	93.27	240.02	74.152	0.16
Fed Glycerol, mg/dL	0.40	0.81	0.214	0.20
Fasted C-Peptide, ng/mL	0.30	0.25	0.070	0.58
Number of Sows	8	8	-	-
Day 5 Leptin, ng/mL HE[2]	3.24	3.27	0.279	0.92
Day 15 Leptin, ng/mL HE[2]	3.24	3.82	0.210	0.07

[1]The 1:1 diet used 8 sows for the epinephrine challenge (fed glucose, NEFA, glycerol) and 7 for the glucose challenge (fasted glucose, C-peptide).
[2]HE = human equivalent

Table 5.6: Plasma concentrations of glucose, NEFA and glycerol during an epinephrine challenge for lactating sows fed diets containing n-6:n-3 FA ratios of 9:1P and 1:1P

	Dietary Treatment (n-6:n-3 fatty acid ratio)		Statistics	
	9:1P	**1:1P**	**SEM**	**P-Value**
Number of Sows	10	8	-	-
Glucose, mg/dL				
Maximum Peak	104.50	104.27	7.725	0.98
Adjusted[1] Maximum Peak	25.58	47.78	9.092	0.09
Total Area Under Curve	10790.0	9971.9	831.85	0.47
Net Incremental Area Under Curve[2]	276.3	2456.0	891.76	0.09
NEFA, uM				
Maximum Peak	281.57	353.12	109.320	0.63
Adjusted[1] Maximum Peak	50.51	56.47	3.730	0.34
Total Area Under Curve	22195.0	30446.0	11811.00	0.61
Net Incremental Area Under Curve[2]	9802.2	-1560.8	5996.13	0.18
Glycerol, mg/dL				
Maximum Peak	1.12	1.26	0.529	0.85
Adjusted[1] Maximum Peak	0.71	0.44	0.379	0.63
Total Area Under Curve	106.4	89.0	47.06	0.80
Net Incremental Area Under Curve[2]	51.9	-18.5	32.74	0.15

[1]Adjusted = adjusted to account for baseline concentration

[2]Net incremental area under curve = total area under curve adjusted for baseline

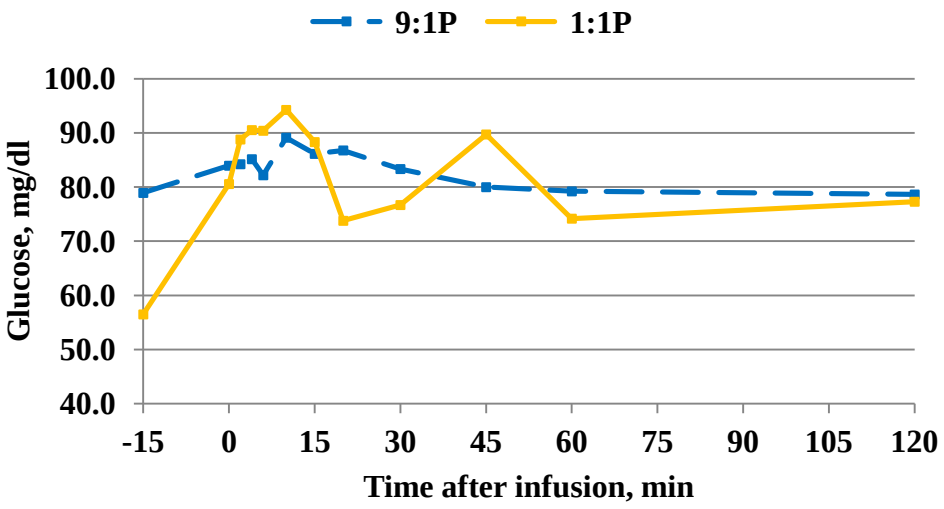

Figure 5.1: Time course response of glucose during an infusion challenge with epinephrine

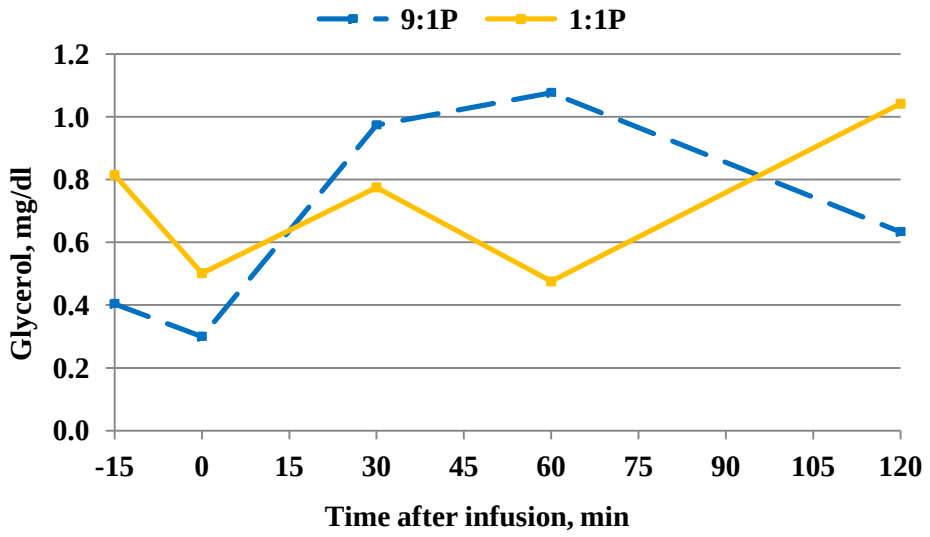

Figure 5.2: Time course response of glycerol during an infusion challenge with epinephrine

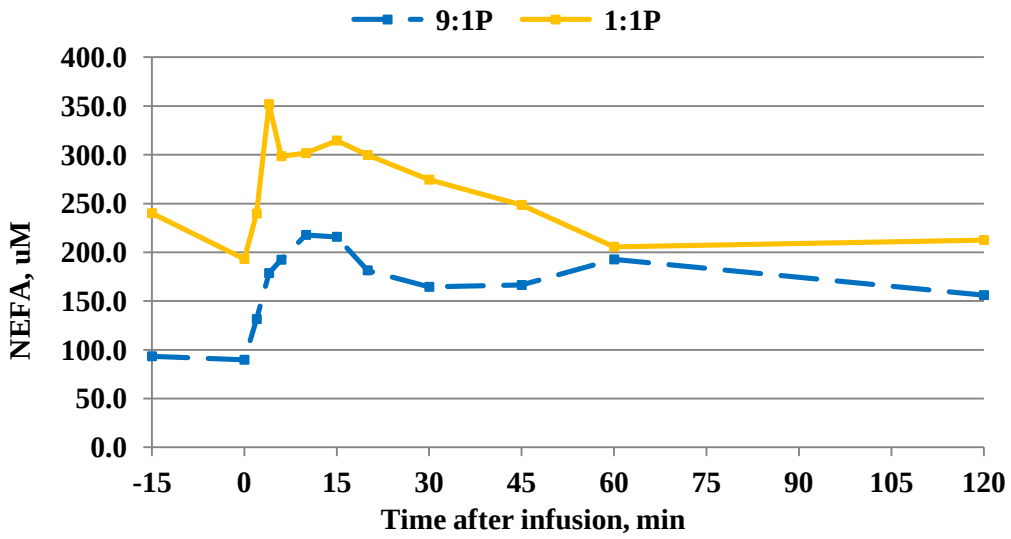

Figure 5.3: Time course response of NEFA during an infusion challenge with epinephrine

Animal performance data for P3 is shown in Table 5.8. There were no effects of diet on lactation length or weaning to estrus intervals (data not shown), or on sow weight changes, as shown in Table 5.8 during lactation ($P > 0.10$). Sows consuming the 5:1P and 1:1P diets had higher backfat thickness prior to farrowing ($P = 0.01$) and at weaning ($P < 0.01$) compared with the other groups. Sows consuming the 5:1F diet lost the most backfat throughout lactation ($P = 0.06$).

Simple correlations between blood parameters and performance parameters were determined (Table 5.9). Leptin was positively correlated with NEFA ($P < 0.10$) and glycerol ($P = 0.10$). Glycerol and NEFA were highly correlated ($P < 0.01$), and were negatively correlated with feed intake ($P < 0.10$). Leptin was not correlated with sow feed intake, but it was negatively correlated to piglet gain ($P < 0.05$), Glycerol and NEFA concentrations were also positively correlated to backfat thickness ($P < 0.05$ for farrowing backfat; $P < 0.10$ for weaning backfat). Leptin was positively correlated to weaning backfat thickness ($P = 0.03$). Overall, this data indicates that sows with greater backfat thickness, i.e., the 1:1P diet group, had increased leptin concentrations, increased plasma NEFA and glycerol (which was correlated to reduced feed intakes), and reduced piglet growth rates.

Table 5.7: Plasma concentrations of glucose and C-peptide during a glucose challenge[1] for fasted, lactating sows fed diets containing n-6:n-3 FA ratios of 9:1P and 1:1P

	Dietary Treatment (n-6:n-3 fatty acid ratio)		Statistics	
	9:1P	**1:1P**	**SEM**	**P-Value**
Number of Sows	10	7	-	-
Glucose, mg/dL				
Maximum Peak	85.89	78.46	5.620	0.33
Adjusted[1] Maximum Peak	21.22	14.92	5.472	0.41
Total Area Under Curve	12371.0	11628.0	1065.41	0.60
Net Incremental Area Under Curve[2]	100.6	-703.3	902.88	0.51
C-Peptide, ng/mL				
Maximum Peak	0.44	0.36	0.094	0.52
Adjusted[1] Maximum Peak	0.14	0.11	0.045	0.63
Total Area Under Curve	50.4	40.4	12.62	0.55
Net Incremental Area Under Curve[2]	-7.8	-20.9	7.88	0.22

[1]Adjusted = adjusted to account for baseline concentration

[2]Net incremental area under curve = total area under curve adjusted for baseline

Table 5.8: Performance of sows consuming differing dietary n-6 to n-3 ratios during reproductive period 3[1]

	Dietary Treatment (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**9:1P**	**5:1P**	**1:1P**	**5:1F**	**SEM**	***P* Value**
Number of Sows	19	19	20	18	19	-	-
Sow Weight, d 110[2], kg	277.8	292.9	297.3	301.3	295.9	8.46	0.29
Sow Weight, farrowing, kg	274.8	290.7	288.4	292.5	285.1	8.65	0.57
Sow Weight, d 7[3], kg	273.0	279.7	289.1	284.5	279.6	9.12	0.69
Sow Weight, weaning, kg	270.2	274.6	282.0	279.7	274.2	8.73	0.85
Total Weight Change, kg/lact	-4.6	-16.1	-6.4	-12.8	-10.9	4.50	0.30
Avg. Daily Weight Change, kg/d	-0.2	-0.6	-0.3	-0.5	-0.4	0.17	0.34
Backfat Thickness, d 110[2], mm	13.4[a]	14.3[ab]	15.3[bc]	15.9[c]	14.7[abc]	0.56	0.01
Backfat Thickness, farrowing, mm	13.6	14.4	15.0	15.8	14.5	0.54	0.06
Backfat Thickness, d 7[3], mm	13.6	14.0	14.4	15.6	14.3	0.54	0.10
Backfat Thickness, weaning, mm	12.5[a]	13.5[ab]	14.1[bc]	15.2[c]	13.0[ab]	0.46	< 0.01
Total Backfat Change, mm/lact	-1.2	-0.9	-0.9	-0.6	-1.5	0.31	0.34
Avg. Daily Backfat Change, mm/d	-0.04[ab]	-0.02[a]	-0.02[a]	-0.02[a]	-0.07[b]	0.02	0.06

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]Period 3 refers to the 3rd reproductive cycle of the sows since initiating diet consumption (approximately 11 months on dietary treatment prior to farrowing)

[2]d 110 refers to the 110th day of gestation

[3]d 7 refers to the 7th day of lactation

Table 5.9: Correlations between blood and performance parameters during period 3[1,2]

	d 5 Leptin	d 15 Leptin	Fasted Glucose	Fed Glucose	C-peptide	NEFA	Glycerol
d 5 Leptin	- -						
d 15 Leptin	NS[3]	- -					
Fasted Glucose	NS	NS	- -				
Fed Glucose	NS	NS	NS	- -			
C-peptide	NS	NS	NS	NS	- -		
NEFA	0.49 0.06	0.46 0.07	0.65 0.01	NS	NS	- -	
Glycerol	NS	0.45 0.10	0.67 0.01	NS	0.54 0.06	0.97 < 0.01	- -
Farrowing Weight	NS	NS	NS	NS	-0.45 0.10	NS	NS
Weaning Weight	NS	NS	NS	NS	NS	NS	NS
Weight Change	NS	NS	-0.47 0.08	NS	NS	NS	NS
Farrowing Backfat	-0.59 0.02	NS	0.50 0.06	-0.46 0.07	NS	0.55 0.03	0.57 0.03
Weaning Backfat	NS	0.55 0.03	NS	-0.53 0.03	NS	0.48 0.06	0.46 0.10
Backfat Change	0.42 0.10	NS	-0.48 0.07	NS	NS	NS	NS
D 0-3 Feed Intake	NS	NS	NS	NS	-0.78 < 0.01	-0.59 0.05	0.61 0.04
Total Feed Intake (lact)	NS	NS	-0.45 0.09	NS	NS	-0.44 0.08	NS
Total ADFI (lact)	NS	NS	NS	NS	NS	-0.45 0.08	NS
Total Piglet Weight Gain	NS	-0.54 0.03	NS	NS	NS	NS	-0.47 0.09
Daily Piglet Weight Gain	NS	-0.68 < 0.01	NS	NS	NS	NS	NS
Live Litter Gain	NS	NS	NS	NS	-0.44 0.10	NS	NS

[1]Top value in each box = correlation coefficient (r)
[1]Bottom value in each box = P Value
[3]NS = Not significant (P > 0.05)

5.5 Discussion

Milk energy output was estimated using equations provided by Noblet and Etienne (1989), which are based on the growth rates of piglets throughout lactation. When litters were standardized to between 10 and 12 piglets, there was no effect of sow diet on piglet ADG, and thus no effects on estimated nutrient and energy output in the milk were observed. This trial aimed to study the effects on high-producing sows, and thus any sow nursing less than 10 piglets was removed from the trial. The final n per diet was 20 sows. Data shows that high-producing sows, regardless of the n-6:n-3 FA ratio they are fed, have similar energy and nutrient outputs in their milk. This indicates that high-producing sows have metabolic adaptations in place to ensure they are able to provide enough nutrients to meet the demands of 10 piglet litters. However it is possible that 10 to 12 piglets per sow was not sufficient to maximize milk output and thus fully test the hypothesis.

Differences in feed intakes seen in this trial may be caused by metabolic signals triggering satiety receptors (such as increases in the hormone leptin), or palatability. Palatability can be defined as the overall acceptance of a feedstuff, with taste being a major constituent (Church, 1977). Rancidity, which may occur when long chain PUFA's are included in animal feed, could lead to reduced feed intake. Lipid peroxidation leading to rancidity occurs when an oxygen free radical interacts with a FA (Halliwell and Chirico, 1993). Polyunsaturated FA's are at a higher risk for peroxidation because a single bond binds hydrogen molecules with a greater affinity than a double bond (Halliwell and Chirico, 1993), resulting in a free radical; the latter can then interact with an oxygen molecule to form a lipid peroxyl radical. If multiple double bonds are present in the FA chain, such as with PUFA's, this process becomes self propagating. The oxidative damage can result in the destruction of fats, and rancidity leading to off-flavours (Halliwell and Chirico, 1993). It is unlikely that rancidity was a factor in the current experiment. The diets contained 0.25g/kg ethoxyquin as an antioxidant, a level greater than commonly found in the literature. Ethoxyquin functions to break the lipid peroxidation reaction chain, by reacting with the peroxyl radical. Diets were made at frequent intervals to prevent long term storage and the associated deterioration. Also, when diets were analyzed for their FA profiles, the long chain PUFA's were present in expected amounts. Although FA rancidity was unlikely in the current trial, palatability issues could still be a factor in the reduction of feed intake seen with the 1:1P

and 5:1F diets. However, sows consuming the 1:1P ratio diet had greater backfat thickness and elevated plasma leptin, indicating that it is more likely that the n-6:n-3 FA ratio of sow diets altered the metabolic signals occurring within the body (as discussed below).

When body fat is used as a source of energy, which typically occurs when an animal is in a state of negative energy balance, the triacylglycerides stored in adipose tissue are broken down into free FA (non-esterified fatty acids, NEFA) and glycerol (Arner, 2003). The FA are transported to the liver where they are oxidized to produce energy (Voet et al., 2008). In lactating sows, a negative energy balance could be caused by a reduction in appetite post-farrowing or by having such a high level of energy output through her milk that she cannot physically consume enough feed to meet her requirements even at maximal feed intake (Whittemore, 1996). In either situation, her energy output exceeds her energy intake, thus putting her into a state of negative energy balance where she must draw on her own body reserves in order to meet her output demands (Jones and Stahly, 1999; Noblet et al., 1990; Vinsky et al., 2006).

Prior to either challenge, on d 5 of lactation, the sows consuming the 1:1P ratio diet may have been in a state of body fat mobilization when compared to those consuming the 9:1P ratio diet. The 9:1P treatment tended to have higher circulating glucose and numerically lower NEFA and glycerol concentration. By d 15, these sows had a tendency for lower plasma leptin. Conversely, the 1:1P sows had decreased circulating glucose and increased NEFA, glycerol and leptin. Total feed intake for the 1:1P diet sows was less than the 9:1P sows throughout lactation. This reduction in feed intake combined with numerically higher circulating levels of NEFA and glycerol may indicate that these sows relied more heavily on body fat reserves to meet energy output demands. The estimated milk energy output based on piglet growth rates was unaffected by dietary treatment, which suggests that despite differences in energy intake, energy outputs were the same, and thus the sows consuming the 1:1P diet were required to rely more on their own energy reserves. Therefore, the problem may not be observed in piglet performance, but rather in sow longevity, return to estrus intervals and her ability to rebreed (Tantasuparuk et al., 2001; Thaker and Bilkei, 2005).

As discussed previously, it is unlikely that palatability was the cause of decreased feed consumption in early lactation for the 1:1P diet sows. Sows in this treatment group had a higher backfat thickness prior to farrowing, during lactation and at weaning. Leptin is a protein hormone produced primarily from adipose tissue (Wylie, 2011). It plays a key role in regulating

energy intake by acting on hypothalamic receptors to inhibit appetite (Campfield et al., 1996), and on regulation of body composition and energy metabolism (Hossner, 1998). Levels of circulating leptin are proportional to the total amount of adipose tissue in the body, which we, and others estimated as backfat depth (De Rensis et al., 2005; Estienne et al., 2000), and is inversely proportional to feed intake during lactation (Estienne et al., 2000). Sows consuming the 1:1P ratio diet had increased circulating leptin levels relative to the 9:1P diet sows, as well as increased backfat thickness. Positive correlations were found between backfat thickness and plasma leptin concentration. Most likely, sows consuming the 1:1P diet had reduced feed intake immediately post farrowing, explaining the subsequent correlations between glycerol and NEFA concentrations (increased plasma leptin was positively correlated with plasma NEFA and glycerol). These findings agree with those of Weldon et al. (1994a), who found that sows with greater fat content pre-farrowing had lower feed intakes post-farrowing. Additionally, negative correlations were present between sow plasma leptin concentrations and piglet weight gain throughout lactation, perhaps due in part to reductions in sow feed intake.

In addition to this, leptin gene expression has been shown to be affected by the PUFA content (Reseland et al., 2001). Gene expression was not measured in the current study; however, it is possible that direct effects of PUFA's on leptin promoter activity, circulating leptin concentrations were altered, in combination with the increased backfat thickness of the 1:1P fed sows. Leptin also reduces FA esterification rates in adipose tissue culture (Ramsay, 2004), which may help to explain why the 1:1P pigs with higher plasma leptin had increased levels of circulating NEFA's. The effects of leptin on adipose tissue metabolism are not present in acute studies, but are evidenced under chronic leptin exposure (Harris, 2000). Sows in our trial were exposed to dietary PUFA alterations for greater than 7 months prior to gestation of this specific section of the trial. Backfat thickness was not different across treatment groups at the onset of the overall trial, and thus changes in backfat seen in P3 occurred over a long time period. Thus, it is possible that long term dietary PUFA manipulation lead to changes in leptin expression and in turn, to changes in FA esterification, as evidenced by increased in circulating NEFA concentrations in 1:1P fed sows.

Sows consuming a diet with a FA ratio of 9:1P had a greater response to the epinephrine challenge, as indicated by a lower niAUC glucose and numerically higher niAUC NEFA and glycerol concentrations. However, the 1:1P ratio sows were possibly mobilizing more body fat

prior to the challenge and they were less sensitive to a dose of exogenous epinephrine than the 9:1P ratio fed sows. The exogenous epinephrine triggered the release of FA's from adipose tissue in the 9:1P fed sows. Similar results were observed by Tilton et al. (1999). They found that pigs which had a reduced response to the epinephrine challenge (10% added tallow) had higher peak levels of circulating glucose. They proposed that the peripheral tissues of these sows may be sparing glucose since they are relying more on circulating FA's for energy. In the current study, the 1:1P fed sows had a reduced response to a metabolic challenge with exogenous epinephrine, and also had increased peak levels of circulating glucose.

In the experiment conducted by Tilton et al. (1999), although plasma NEFA concentrations increased in response to the epinephrine challenge, glycerol did not increase. This leads to the conclusion that the changes seen were due to alterations in the clearance rates of NEFA from circulation, and were not due to a change in adipose tissue responsiveness. We observed numerical increases in NEFA and glycerol occurred, possibly indicating that the adipose tissue of sows fed the 9:1P n-6:n-3 FA ratio diet was more responsive to the epinephrine challenge, whereas those fed the 1:1P ratio perhaps had desensitized tissue due to the pre-challenge state of body fat mobilization. Sows with more responsive adipose tissue would be more readily able to mobilize their body fat stores during a period of stress or energy balance challenge, and thus should be able to better cope with the negative energy balance which occurs in early lactation. Overall, it appears that sows consuming diets with reduced n-6:n-3 FA ratios were in a state of metabolic energy usage, which reduced the sensitivity of their adipose tissue. The sows fed a higher n-6:n-3 ratio reached a greater level of lipolysis based on the niAUC of NEFA, glycerol and glucose when challenged.

When sows were presented with an exogenous glucose challenge, no diet effects were observed. Both Tilton et al. (1999) and Coffey et al. (1987) reported no dietary effects on plasma insulin concentration due to challenge. In the current experiment, the glucose challenge caused the expected increase in plasma glucose and C-peptide (pre-insulin), but there were no differences between diets. Glucose concentration rose to a level similar to the baseline values obtained when the sows were in the fed state, indicating that our challenge model may not have pushed sows beyond the capacity of an insulin response and that the insulin response was unaffected by the FA ratio in the diet. The experimental diets did not have an effect on insulin

sensitivity of sow tissues, but did affect the responsiveness of adipose tissue when a negative energy balance model was used.

5.6 Conclusions

In conclusion, reducing the n-6:n-3 FA ratio in sow diets affects sow performance. Piglet performance and sow feed intake were similar between the 5:1P and control treatment groups, while sows consuming a plant based ratio of 1:1 or a fish based ratio of 5:1 had reduced feed intakes. Metabolic adaptations of the sows were measured in the 9:1P and 1:1P fed groups and results show that sows fed the 1:1P ratio diet were in a state of negative energy balance relative to the 9:1P pigs throughout early lactation. There were no differences between diets on piglet performance, and thus on estimated milk energy and nutrient outputs, and with the exception of the fish based diet, there were no effects on piglet growth rates. This implies that sows can compensate for changes in feed intake through body fat mobilization, ensuring that their offspring are provided with an adequate supply of energy and nutrients for growth.

A reliance on the use of body fat reserves could have negative long term effects on the sow, leading to a reduced reproductive lifespan in the herd and increased cost of production. Combining the production data for all 5 dietary groups with the metabolic data of the 9:1P and 1:1P diet sows, when the n-6:n-3 ratio becomes too low, negative effects on sow performance are seen, as described above. A typical gestation or lactation corn, soybean meal diet (containing approximately 70% corn and 20% soybean meal) has an n-6:n-3 fatty acid ratio between 25:1 and 20:1. Sows consuming the 5:1P diet had similar performance to those consuming the control diet; however, we do not know how their metabolic performance compares to the 9:1P and 1:1P diet groups.

6 EFFECTS OF ALTERING THE OMEGA-6 TO OMEGA-3 FATTY ACID RATIO IN SOW DIETS ON THE INFLAMMATORY RESPONSES OF THEIR OFFSPRING POST-WEANING WHEN CHALLENGED WITH *E. COLI* LIPOPOLYSACCHARIDE

6.1 Abstract

This experiment was designed to test the hypothesis that offspring born to sows fed reduced omega-6 (n-6) to omega-3 (n-3) fatty acid (FA) ratios have a diminished inflammatory response post-weaning. The hypothesis is based on the notion of the anti-inflammatory properties of n-3 FA's relative to n-6 FA's, and thus piglets raised by sows consuming diets with reduced n-6:n-3 ratios would have decreased acute inflammatory responses post-weaning during an *E. coli* based lipopolysaccharide (LPS) challenge. Specifically, the objective was to determine the dietary effects on febrile and pro-inflammatory cytokine responses one week post-weaning.

Piglets (n = 100, 20/diet) were weaned from sows consuming 1 of 5 wheat and barley based diets. The diets contained 5% crude fat and varied n-6:n-3 ratios. The treatment groups consisted of a control diet (tallow based, low in PUFA), 3 diets with plant oil based n-6:n-3 ratios (9:1P, 5:1P, and 1:1P) and a 5:1 fish oil diet (5:1F). Sows remained on these diets for 2 reproductive cycles and piglets were weaned from the 2^{nd} cycle (d 26 ± 2 of lactation) for this experiment. The FA profile of the milk reflected the sow diets, with ratios of 7.5:1, 4.5:1, 1.5:1 and 3:1 for the 9:1P, 5:1P, 1:1P and 5:1F diets respectively. Post-weaning, piglets were randomized, within diet, to a challenge control group (saline injected) or to a LPS injected group (n=10/challenge·diet^{-1}). Piglets were given 6 days to acclimate to their new environment prior to the immune challenge, and all piglets were fed a common production starter diet throughout the acclimation and challenge periods. Saline or LPS was injected on d 7 post-weaning. Rectal temperatures were recorded at 0, 1, 2, 3, 4, 5, 6, 12 and 24 hrs post injection and blood samples were collected at 0, 2, 6 and 12 hrs post injection for cytokine and blood urea nitrogen (BUN) analysis.

Body temperature, interleukin (IL)-1β, IL-8 and tumor necrosis factor (TNF)α, were higher in the challenged pigs ($P < 0.05$) indicating that an injection of 15 ug/kg body weight LPS was effective in generating an inflammatory reaction. Additionally, injecting LPS caused decreased feed intake and reduced growth of the piglets ($P < 0.01$). Piglets raised under sows

consuming the 1:1P diet had elevated temperatures regardless of challenge (P = 0.01). Piglets from the 1:1P and 5:1F diet groups had a numerically greater IL-8 response to the LPS challenge when compared with piglets from sows fed the other diets, and also had numerically greater febrile responses to the LPS challenge. Dietary treatment did not affect BUN concentrations during the challenge period.

Weanling pigs born from sows consuming different n-6:n-3 FA ratios had differential responses to an LPS induced inflammatory reaction. When a dietary ratio of 1:1 was reached, piglets had elevated body temperatures at baseline and higher peak body temperatures during the inflammatory challenge. Piglets raised by sows consuming the other diets did not have altered febrile responses prior to, or throughout the challenge period.

Keywords: cytokine, inflammatory response, lipopolysaccharide, omega-3, omega-6, piglet

6.2 Introduction

Weaning is a stressful period in a piglet's life. Piglets are removed from the sow, moved to a new room either in the same barn or transported offsite, mixed with unfamiliar pen mates and begin consuming a new, unfamiliar, solid diet (Patience et al., 1995). These stressors (social, environmental and nutritional) can contribute to the induction of an inflammatory response, which may affect piglet performance in the nursery. Although a certain degree of immune response is beneficial, an over-production of inflammatory cells can become detrimental, leading to reduced protein synthesis and even muscle degradation (Zhan et al., 2009).

Many nutritional strategies have been implemented with the goal of improving piglet performance post-weaning. Some of these strategies include the use of creep feed in the farrowing room and the use of highly palatable protein sources or novel ingredients in starter diets to encourage piglets to eat immediately post-weaning. Other strategies focus on sow diets to provide 'beneficial properties' to milk, and subsequently to the offspring. The use of omega-3 (n-3) fatty acids (FA) is of growing interest to pork producers due to their anti-inflammatory properties.

Omega-3 and omega-6 (n-6) FA's are long chain polyunsaturates (PUFA). The n-3 and n-6 FA's are similar in structure, but differ in the location of their double bonds. These slight modifications in structure lead to differences in the biological activity of molecules produced from the FA precursor (Lands, 1992). Molecules produced by the n-6 FA's are considered pro-inflammatory and the n-3 products anti-inflammatory or less-inflammatory than the n-6 products (Palmquist, 2009). The n-3 FA's can alter the body's release of pro-inflammatory cytokines (Carroll et al., 2003; Chavali et al., 1998; Korver and Klasing, 1997). Cytokines are proteins secreted by immune cells in response to stimuli, which assist in regulating the inflammatory response (Tizard, 2009). Some of the most important pro-inflammatory cytokines are tumour necrosis factor (TNF-α), interleukin (IL)-1, IL-6 and IL-8 (Tizard, 2009). The ability of n-3 FA's to alter cytokine production may contribute to their anti-inflammatory properties, and may be beneficial in alleviating the inflammatory response generated at the time of weaning.

Overall, the objective was to determine if the inflammatory responses of piglets post-weaning could be lessened by reducing the n-6:n-3 FA ratio in sow diets throughout gestation and lactation. It is hypothesized that piglets raised by sows consuming diets with reduced n-6:n-3

ratios have reduced inflammatory responses post-weaning during an *E. coli* based lipopolysaccharide (LPS) challenge, due to the anti-inflammatory properties of n-3 FA's. Specifically, the objective was to determine the effects of reducing sow n-6:n-3 dietary ratios on febrile and pro-inflammatory cytokine responses of their offspring one week post-weaning during a 24 hour inflammatory challenge.

6.3 Materials and Methods

Sow aspects of this trial were the same as described in Chapters 4 and 5 for treatment groups and management practices.

6.3.1 General

This experiment was conducted at the Prairie Swine Centre Inc., in Saskatoon, Saskatchewan, Canada. The study was approved by the University of Saskatchewan's Animal Research Ethics Board (UCACS #'s 19970021 and 20090094), and adhered to the Canadian Council on Animal Care guidelines for humane animal use (CCAC, 1993). Animals were housed in temperature-controlled rooms according to the thermoneutral zone for the specific age and stage of reproduction (Zhang, 1994), and lighting was maintained on a 12 h light:dark cycle (07:00 – 19:00). All pigs were a commercial cross-bred (Camborough Plus females x C3378 sires, PIC Canada Ltd., Winnipeg, Manitoba, Canada). The flaxseed meal (FSM) and flax oil (FO) were obtained from a commercial company (Vandeputte S.A., Mouscron, Belgium). The chemical composition of this FSM product has been previously reported by Eastwood et al. (2009).

6.3.2 Animals and Housing

A total of 100 piglets (barrows, 10.3 ± 1.4 kg) obtained from the sows described in Chapter 4 were used in the LPS challenge study. Piglets were weaned from sows fed one of 5 dietary treatments (n = 20 piglets per sow diet) and housed in a nursery located within the same

barn as the sows. Sows had been on the experimental diets for approximately 6 months prior to farrowing and piglets were weaned on d 26 ± 2 of lactation.

The nursery was equipped with 6 identical rooms, each with 16 pens. The pens measured 213 cm long by 122 cm wide and had plastic coated expanded metal floors, PVC siding and metal front gates. For experimental purposes, pens were divided in half (105 cm long by 122 cm wide), with each half containing a feeder and nipple drinker for *ad libitum* access.

A detailed description of sow management and housing can be found in Chapters 4 and 5. Briefly, A total of 150 sows (240 ± 33 kg, parity 0 to 4 at experiment initiation) were utilized (n=30/treatment) in a completely randomized design. Treatment groups were balanced across parity, and sows were assigned to diets based upon their expected farrowing dates. Throughout gestation all animals were group housed in a free access stall system. On d 110 of gestation sows were moved from the group housing gestation facility to a farrowing room equipped with 16 individual farrowing crates. Heat lamps were provided for the piglets.

6.3.3 Treatments and Feeding

A full description of diets can be found in Chapter 4 and are shown in Table 4.1a/b and Table 4.2a/b. Five sow dietary treatments were used for this experiment, each divided into a gestation and a lactation ration. Diets were formulated based on their digestible oil content to contain equal amounts of crude fat (5%), and were balanced for net energy and digestible essential amino acids according to NRC (1998) recommendations for gestating and lactating sows. Diets were wheat and barley based, and contained supplemental vitamins, minerals and amino acids to meet requirements. Oil sources were supplemented at varied levels in order to adjust the ratio of n-3 to n-6 FA's without altering the total fat content. The treatment groups consisted of a control diet (tallow based, low in PUFA), 3 diets with plant oil based n-6:n-3 ratios (9:1P, 5:1P, and 1:1P) as well as a 5:1 fish oil diet (5:1F).

6.3.4 Experimental Procedure

Sows began consumption of their assigned diets 5 weeks prior to farrowing (± d 80 of gestation). Sows were fed the gestation diets until d 110 of gestation, at which point they were switched to the lactation ration. Following weaning, sows returned to their respective gestation diet. Piglets used for this experiment were weaned from sows that had completed their 2nd lactation after initiation of diet consumption (Figure 6.1).

Prior to starting actual data collection, a few pigs were randomly selected to be treated with LPS to determine the dose required to generate a measurable, yet non-lethal inflammatory response. Based on the literature and personal communication with Dr. Nicolas Gabler (Iowa State), this dose would be between 10 and 15 μg/kg BW of *E. coli* 055:B5 based LPS (catalogue # L2880, Sigma-Aldrich Canada, Oakville, Ontario). The pre-trial indicated a dose of 15 μg/kg was suitable.

The LPS challenge study used 100 piglets (n = 20/sow diet). Due to weekly piglet availability, the challenge was conducted over a period of 6 weeks. Piglets were all barrows and of approximately equal starting body weights (10.3 ± 1.4 kg). Each week, 2 to 4 piglets per sow were selected from available sows up to a maximum of 36 piglets (to ensure timed collections would not overlap). Piglets represented average body weight within their litters. Post-weaning, all of the piglets were fed the same diet (commercial starter as per normal production practices) and were housed in groups of 2 per pen. All piglets were housed with an unfamiliar pen-mate of similar size, thus ensuring that each piglet was subjected to the same stress of mixing. Piglets were acclimated to their new environment and solid feed for a period of 6 days, followed by a 24 h challenge period. In order to reduce the effects of handling stress on the piglets, all piglets within each of the pre-selected litters were handled briefly a minimum of 4 times per week for 2 weeks prior to the trial, and were placed into a recumbent position to mimic blood collection as well as having a rectal thermometer inserted.

Piglets within each sow dietary group were randomized to either a control treatment (saline injection) or a LPS treatment (injection of 15μg LPS/kg BW). For the 24 h challenge, even numbers of piglets were selected from litters, and were divided between saline and LPS treatments. Piglets within an individual pen received the same treatment (saline or LPS) to ensure that sick pigs did not undergo the additional stress of being housed with a healthy piglet.

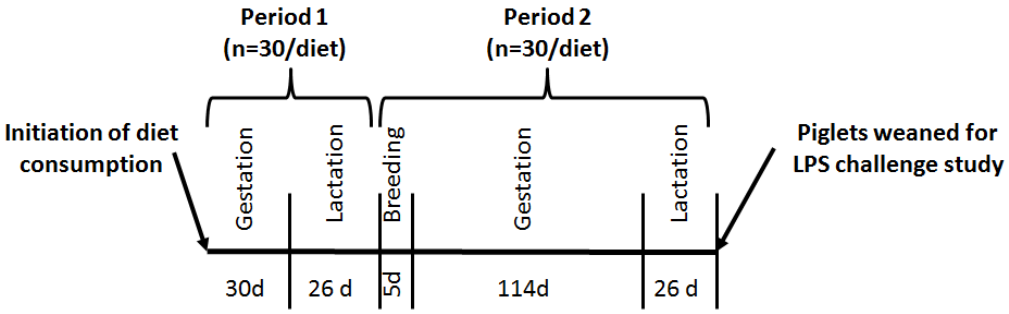

Figure 6.1: Experimental timeline of sow feeding and obtaining piglets for the lipopolysaccharide (LPS) challenge study

Throughout the 24 h challenge period, feed disappearance for each pen was recorded and all pigs were weighed at time 0 and time 24 h. At time 0 h, rectal temperatures were recorded and a pre-challenge blood sample was drawn from each piglet. This was immediately followed by an injection of either LPS in saline or an equivalent amount of saline only. Rectal temperatures were recorded every hour for the first 6 hours post injection and then at 12 and 24 hours. Blood was collected by jugular venipuncture into evacuated blood tubes containing heparin as an anticoagulant at time 0, 2, 6 and 12 h for analysis of pro-inflammatory cytokines and blood urea nitrogen. To facilitate collections the pens were started over a 1 hour period.

6.3.5 Analytical Methods

Proximate analyses of sow diets were performed by a commercial laboratory (Central Testing Laboratory Ltd (Winnipeg, Manitoba, Canada). Measures included dry matter (method 930.15; AOAC, 1990), ash (method 923.03; AOAC, 1990), nitrogen (Leco Analyzer, St. Joseph, MI), crude fat (ANKOM XT20), crude fibre (AOCS Ba6a-05), acid detergent fibre (ANKOM 08-16-16), lignin (ANKOM 3/98), calcium and phosphorus (methods 968.08 and 935.13A; AOAC, 1990).

Sow diets were also analyzed for their FA profile using GLC (Agilent 6890 system with Agilent ChemStation Software; Agilent Technologies, Mississauga, Ontario, Canada) as described in Chapter 4. Briefly, direct FA methylation was performed according to the procedure of O'Fallon et al. (2007). Non-methylated C13:0 (Nu-Chek Prep Inc, Elysian, MN) was used as the internal standard, and all other chemicals were GLC grade (Sigma-Aldrich Inc., St. Louis, MO). Fatty acid methyl ester (FAME) samples were compared with a standard mixture containing a wide array of FAME's ranging from C8:0 to C24:1 (GLC-68-D, GLC-97 and U-62-M; Nu-Chek Prep Inc, Elysian, MN) using a GLC program slightly modified from the procedure described by O'Fallon et al. (2007) and a Supelco fused silica capillary column SP 2560 (Sigma-Aldrich Inc., St. Louis, MO). The instrument was set for a 1.0 µl injection split at a ratio of 30:1. The injector set points were a temperature of 260^{o}C, pressure of 40.24 psi, and a total flow for the carrier gas (helium) of 37.5 mL/min. The initial oven temperature was 140^{o}C and held for 5 min. Temperature was then ramped up at a rate of 4^{o}C/min to a maximum of 240^{o}C and held for 15 min. The total analysis run time was 45 min. A flame ionization detector was utilized for

detection, with the heater set at 250^{o}C, hydrogen flow of 40 mL/min, air flow of 450 mL/min and helium flow of 45 mL/min.

Plasma samples were analyzed for TNF-α, IL-1β, IL-6, IL-8 and BUN. Cytokines were determined for 50 piglets (10 randomly selected per sow diet) for 0, 2, 6 and 12 h samples. Samples were shipped on dry ice to Aushon Biosystems (Billerica, MA) for analysis using their SearchLight® Technology with a custom multiplex immunoassay kit. Blood urea nitrogen was analyzed for the 0, 6 and 12 h samples of all piglets using a commercially available colorimetric analysis kit (QuantiChrom Urea Assay Kit, BioAssay Systems, Hayward, CA). The analysis had linear detection range of 0.08 to 100 mg/dL and an intra-assay CV of 9.5%.

6.3.6 Statistics

All data (rectal temperatures, body weight changes and blood parameters) were statistically analyzed using the PROC MIXED function of SAS (version 9.2; SAS Inst. Inc., Cary, NC). Pre-challenge and challenge feed intakes for each pen were analyzed as a completely randomized design to determine the effects of LPS or saline injection on feed consumption. Treatment (LPS or Saline) was the fixed effect, with pig as the random effect. Individual piglet ADG throughout the 24 h challenge period, as well as time 0 rectal temperatures and BUN concentrations were analyzed as a split plot design. Sow was considered the main plot, with sow diet as the main plot factor (control, 9:1P, 5:1P, 1:1P and 5:1F), and piglet within pen was the subplot, with challenge as the subplot factor (LPS vs. Saline injection). For this model, the effects of diet, challenge and the diet by challenge interaction were considered fixed, with the effect of sow being the random factor.

Time course data for rectal temperatures and BUN were analyzed as a split plot design with repeated measures. As described, sow was the main plot, with sow diet as the main plot factor (random effect), and piglet was the subplot with challenge as the subplot factor. The fixed effects were diet, challenge, time and all 2 and 3-way interactions. For the repeated measures component, a compound symmetry structure was best fit for rectal temperatures and heterogeneous Toeplitz was the model of best fit for BUN due to equal time spacing. Models were selected based on the Akaike information criteria (AIC) and Bayesian information criteria

(BIC) values. Where discrepancies were present amongst which value was lowest, BIC was chosen as the deciding factor as suggested by Quinn and Keough (2002).

Since cytokines were only analyzed for half of the piglets, data was analyzed as a completely randomized design with a 5 by 2 factorial arrangement of treatments (5 sow diets x 2 challenges). Repeated measures were used within the factorial for analysis over the entire challenge time period. Diet, challenge, time and all 2 and 3-way interactions were the fixed effects, with pig as a random effect. An unstructured model was selected as the best fit for IL-1β, IL-8 and TNFα whereas heterogeneous compound symmetry was the model of best fit for IL-6.

The split plot and factorial models allowed for comparison of the effects of sow diet and LPS challenge, as well as for their interactions. Including the repeated measures allowed for the additional comparison of time, and its interactions with diet and challenge. For all data, significance was defined at $P \leq 0.05$, with tendencies defined at $P < 0.10$. Where applicable, the statistical model included Tukey's honestly significant difference test for means separation and room (week) was included as a block.

6.4 Results

As described in Chapter 4, the FA analysis of late lactation milk samples revealed that the n-3:n-6 profiles were similar to those of the sow diets, with ratios of 7.5:1, 4.5:1, 1.5:1 and 3:1 for the 9:1P, 5:1P, 1:1P and 5:1F diets respectively. Throughout lactation piglets were consuming milk with a FA n-6:n-3 ratio similar to the diet ratio consumed by their dam.

During the experiment, one piglet died 4 hours post LPS injection. It is possible that this pig had an underlying illness or infection as no other pigs died or failed to recover from the LPS challenge within 24 h.

The effects of sow diet on piglet feed intake could not be measured. Post-weaning, piglets were housed with an unfamiliar pen mate, and may have come from sows consuming different dietary treatments. Since all piglets were fed a common diet, this did not affect biological measures, but did not allow for the determination of sow diet effects on piglet feed intake. There was no effect of challenge treatment (LPS or saline) randomization on feed intakes ($P > 0.5$)

during the acclimation phase. During the 24 hour challenge period, ADFI was significantly reduced for piglets injected with LPS (230 g) relative to the saline controls (369 g; $P < 0.01$). Feed intake data is shown in Table 6.1.

Piglet ADG during the 24 h challenge period was unaffected by the sow dietary treatment ($P > 0.05$). As expected, and similar to feed intake during the challenge period, ADG's were reduced in piglets that received the LPS injection (64 g) vs. the saline injection controls (235 g; $P < 0.01$). No diet by challenge interactions were observed for piglet growth. Experimental effects on ADG are outlined in Table 6.2.

Piglet rectal temperature was affected by diet ($P = 0.01$), time ($P < 0.01$) and by the LPS challenge ($P < 0.01$). The effects of sow diet on piglet body temperature throughout the challenge period post-weaning are shown in Figure 6.2. Piglets raised under sows consuming the 1:1P diet had elevated temperatures (39.9^{o}C) relative to those raised by sows consuming the 9:1P (39.5^{o}C) and 5:1P (39.5^{o}C) diets, with the control diet (39.6^{o}C) and 5:1F diet (39.7^{o}C) producing piglets with intermediate body temperatures. Figure 6.3 shows the temporal pattern following the LPS injection. Similar to feed intake and weight gain, the presence of LPS elicited significant responses in fever production. The overall responses of piglets from each diet and challenge group over the full 24 hour period are illustrated in Figure 6.4.

The challenge by time interaction for body temperature was significant ($P < 0.05$; Figure 6.4). Additionally, the diet by LPS challenge interaction tended to differ for body temperature ($P = 0.11$), with piglets originating from the 1:1P fed sows having greater febrile responses LPS compared with piglets raised by sows consuming the other diets (Figure 6.5).

Time 0 h (baseline) cytokines (IL-1β, IL-6, IL-8 and TNFα) were unaffected by dietary or challenge treatments ($P > 0.05$). Since baseline values were the same for all piglets, any responses can be attributed to the dietary background of the piglet interacting with the inflammatory challenge. The P values for effects of sow diet, challenge, time and all interactions for each cytokine are presented in Table 6.3. There were no effects of diet on cytokine responses of piglets. IL-1β and IL-6 tended to be increased in the LPS challenged piglets ($P = 0.06$), whereas IL-8 ($P = 0.02$) and TNFα ($P < 0.01$) were elevated by the LPS challenge. The challenge by time interaction was also important, with piglets spiking an IL-1β, IL-8 and TNFα response by hour 2 and returning to normal by hour 12 post LPS ($P < 0.01$).

Table 6.1: Average daily feed intakes of piglets (pens)[1] during a 6 day acclimation period post-weaning (pre-challenge ADFI) and during a 24 hour challenge period (challenge ADFI) when randomized to receive an injection of saline (control) or lipopolysaccharide (LPS)

	Challenge		Statistics	
	Saline	**LPS**	**SEM**	**P Value**
Number of Pens[1]	25	25	-	-
Pre-Challenge ADFI, g/d	207	193	10.0	0.33
Post-Challenge ADFI, g/d	369	230	17.8	< 0.01

[1]Feed intakes were determined for each pen which contained 2 piglets

Table 6.2: Average daily gains of piglets[1] raised by sows consuming varied n-6:n-3 FA ratios when injected with saline (control) or lipopolysaccharide (LPS) during a 24 hour challenge period

	Challenge		Statistics[2]	
	Saline	**LPS**	**SEM**	**P Value**
Number of Pens[1]	25	25	-	-
ADG, g/d	235	64	31.9	< 0.01

[1]Values presented are the average value of 2 individual pigs per pen

[2]No effect of diet or diet by challenge interactions were observed (P > 0.05)

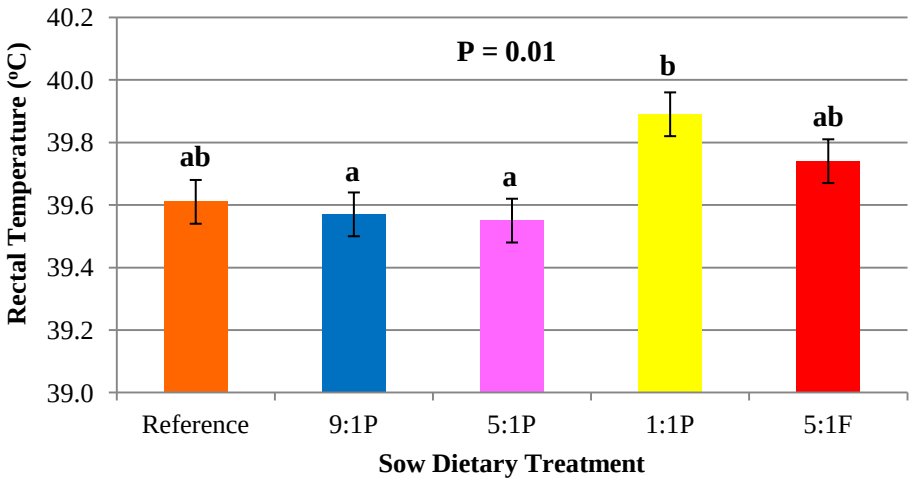

Figure 6.2: Effect of sow dietary treatment on piglet rectal temperature post-weaning during a 24 h challenge, regardless of challenge group (saline or lipopolysaccharide injection) (mean ± SEM). Bars without common superscripts differ (P ≤ 0.05)

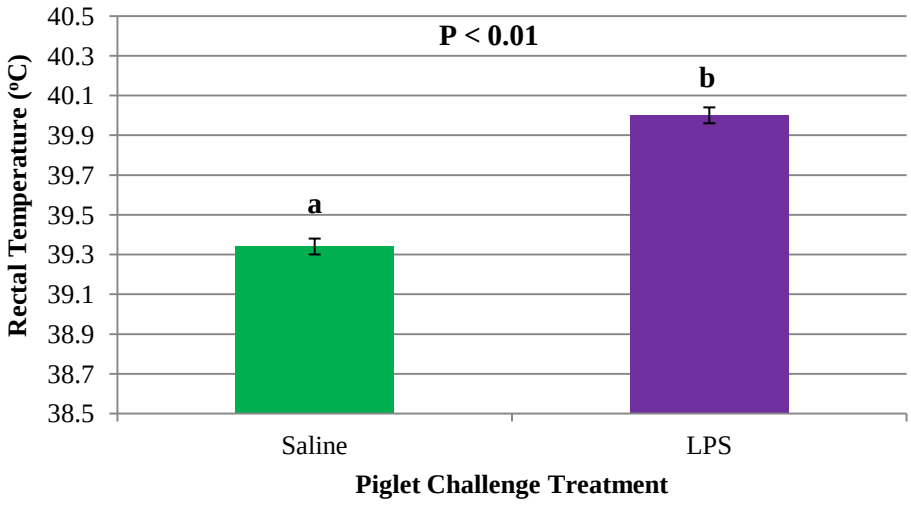

Figure 6.3: Effect of piglet challenge group (saline vs. lipopolysaccharide injected) on rectal temperature during a 24 h challenge, regardless of which sow dietary treatment group they originated from (mean ± SEM). Bars without common superscripts differ (P ≤ 0.05)

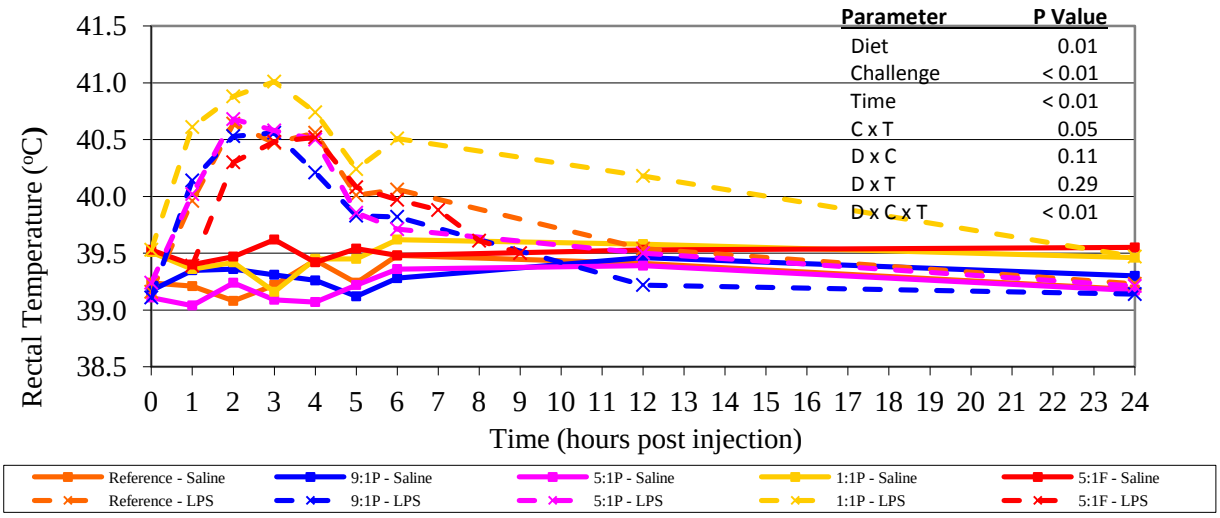

Figure 6.4: Average rectal temperatures of pigs treated with lipopolysaccharide (LPS) or saline after being raised by sows consuming varying n-6 to n-3 fatty acid ratios (effect of diet at time zero prior to challenge; $P > 0.05$)

There were no significant diet by challenge interactions observed; however, there was large variability in plasma cytokine measures between pigs. It is possible that with increased piglet numbers, differences may have been seen. As an example, Figure 6.6 shows the diet by challenge interaction of IL-8. Piglets from the 1:1P and 5:1F diet groups had numerically greater IL-8 responses to the inflammatory challenge when compared to piglets from the control, 9:1P and 5:1P diets ($P = 0.18$).

Blood urea nitrogen concentrations were elevated in piglets who received the LPS injection relative to the saline controls ($P < 0.01$). Additionally, BUN concentrations were affected by time ($P < 0.01$), with the highest values occurring at time 0 h (38.5 mg/dl), intermediate at time 6 h (33.1 mg/dl) and lowest at time 12 h (29.5 mg/dl). No differences were observed in BUN concentration due to dietary background and no diet by challenge interaction was present, indicating that pigs consuming all diets had similar responses to the LPS challenge. Blood urea nitrogen results are shown in Table 6.4.

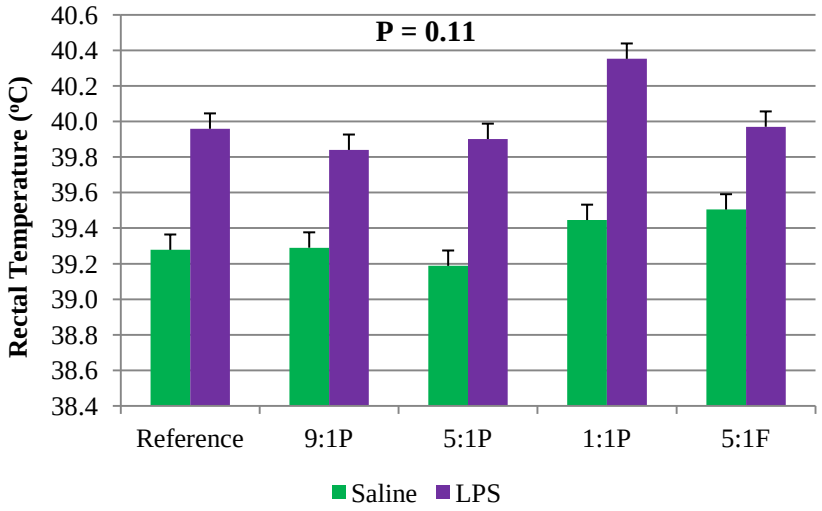

Figure 6.5: Diet by challenge interaction of body temperature in piglets (mean ± SEM) for piglets injected with saline or lipopolysaccharide (LPS) when raised by sows consuming varied n-6 to n-3 ratios

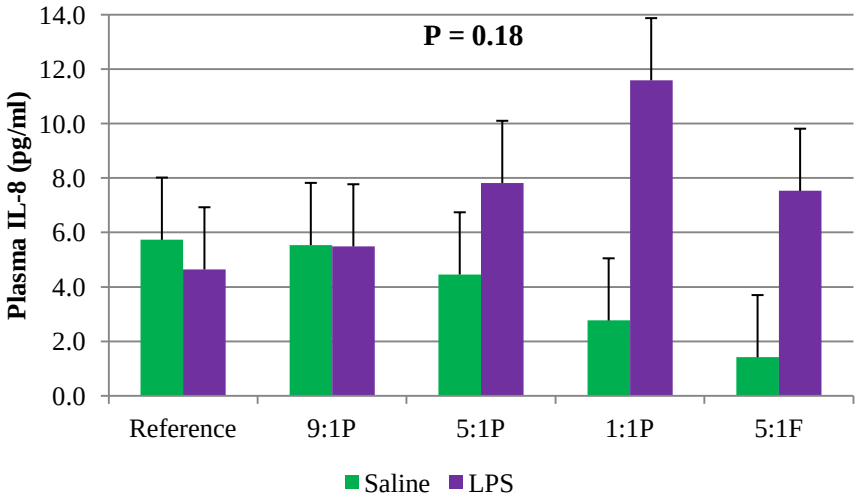

Figure 6.6: Diet by challenge interaction of plasma interleukin (IL)-8 concentration (mean ± SEM) for piglets injected with saline or lipopolysaccharide (LPS) when raised by sows consuming varied n-6 to n-3 ratios

Table 6.3: P-values for each parameter and their interactions for plasma cytokines when piglets were raised by sows consuming varied n-6 to n-3 fatty acid ratios and injected with saline or lipopolysaccharide over a 12 hour collection period[1,2]

	P-Values						
	Diet[3]	**Challenge[4]**	**Time[5]**	**Diet x Challenge**	**Diet x Time**	**Challenge x Time**	**Diet x Challenge x Time**
IL-1β[6]	0.82	0.06	< 0.01	0.59	0.98	< 0.01	0.88
IL-6	0.73	0.06	0.12	0.73	0.82	0.08	0.81
IL-8	0.81	0.02	< 0.01	0.18	0.12	< 0.01	0.14
TNFα[6]	0.54	< 0.01	< 0.01	0.47	0.74	< 0.01	0.72

[1]No effects of diet, challenge, or their interactions were observed for any measured cytokine at time zero prior to pigs receiving a dose of LPS or saline

[2]Cytokine analysis was conducted on 5 piglets per dietary treatment group

[3]Sow diets included a control diet and n-6:n-3 FA ratios of 9:1P, 5:1P, 1:1P and 5:1F

[4]Challenge refers to piglets receiving an injection of saline (control) or *E. coli* lipopolysaccharide (LPS)

[5]Samples were collected at time 0 (pre-injection), 2, 6 and 12 hours

[6]IL = interleukin; TNFα = tumor necrosis factor α

Table 6.4: Effect of inflammatory challenge and time on piglet[1] blood urea nitrogen during the 24 hour challenge period

	Challenge		Statistics[2,3]	
	Saline	**LPS**	**SEM**	**P Value**
BUN (mg/dl)	31.36[a]	36.03[b]	1.225	< 0.01

	Time (hr post injection)			Statistics	
	0	**6**	**12**	**SEM**	**P Value**
BUN (mg/dl)	38.46[a]	33.13[b]	29.49[c]	1.071	< 0.01

[a-c]Within a row, means without a common superscript differ ($P \leq 0.05$)

[1]BUN was analyzed for a total of 50 piglets per challenge group (10 per diet per challenge)

[2]No effect of diet or diet by challenge interactions were observed ($P > 0.05$)

[3]No differences were detected in BUN concentrations across treatment groups (diet or challenge) at time zero prior to injection

6.5 Discussion

The objective of this study was to determine if reducing the dietary n-6:n-3 FA ratio for pregnant and lactating sows would alter the inflammatory responses of their offspring post-weaning when challenged with LPS. The aim was to develop a new strategy for reducing the inflammatory responses of piglets post-weaning. Social, nutritional and environmental stressors that piglets are subjected to in the nursery may result in an over stimulation of the immune system. Many previous strategies for reducing the inflammatory response have targeted the piglet at the time of weaning; for example, including highly palatable ingredients in starter feeds to encourage intake. This experiment attempted to mitigate the potential over-activation of the inflammatory response in piglets through the sow's nutrition.

There have been many studies showing that altering sow diets can affect the performance of their offspring while nursing (Leonard et al., 2010b; Mateo et al., 2009; Mateo et al., 2008; Rooke et al., 2000; Shen et al., 2011; Smits et al., 2011; Webel et al., 2004). Most of these studies however, do not follow the piglets through the weaning period to determine if and how dam nutrition can impact piglet responses to stressors encountered in the nursery. Piglets often go off feed when they first enter the nursery, and using the sow as a source to impact post-weaning performance is an attractive option.

The use of an LPS challenge model is well established in many animals (Rakhshandeh and de Lange, 2012), including pigs. It generates a moderate inflammatory response triggering activation of the innate immune system (Carroll et al., 2003). The characteristic responses seen with an LPS challenge including increased circulating pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα and the presence of a fever, decreased feed intake and reduced growth (Webel et al., 1997). It is for this reason that the LPS challenge model can be used to mimic the occurrence of stress related immune responses (Curfs et al., 1997) such as those which occur at the time of weaning. Additionally, LPS is a non-pathogenic endotoxin, and thus can be used in biosecure herds without eliciting disease transmission between animals (Carroll et al., 2003).

The reductions in ADG and ADFI observed in LPS challenged pigs in the current experiment, in conjunction with elevated febrile responses, are a clear indication that the inflammatory challenge model was successful. Febrile responses had returned to baseline after the 24 h period, and cytokine levels had also returned to baseline by 12 h post injection,

signifying that the inflammatory response was of short duration. In addition, an effect of LPS challenge, time and their interactions were observed for Il-1β, IL-6, IL-8 and TNFα, showing that our challenge model was able to generate a time-course inflammatory response in the piglets, similar to that observed by Webel et al. (1997), who observed elevated IL-6 and TNFα post LPS injection.

As expected, piglets receiving LPS treatment had reductions in ADG and ADFI throughout the 24 hour challenge period. The effects of the n-6:n-3 FA ratio in sow diets on piglet feed intake during the challenge could not be measured due to the experimental design. Unfortunately, piglets were required to be housed in groups of two, and all piglets were subjected to the stress of mixing as per normal weaning practices within a hog facility. This meant that piglets were then housed with a partner who many have originated from a sow consuming a different dietary treatment as not all dietary treatments were available in equal numbers each week. In all cases, a pen of two piglets was assigned to either LPS treatment or saline treatment, allowing for the comparison of immune challenge, but not diet interactions for ADFI parameters.

Despite this limiting issue in the design of the experiment, individual piglet weights were collected and thus the effects of sow treatment on offspring ADG throughout the inflammatory challenge were determined. There were no differences in piglet ADG's based on sow dietary treatment. Although there are very few studies in the literature addressing the effects of sow diet on the responses of piglets to an immune challenge post-weaning, Carroll et al. (2003) found that including fish oil into piglet diets for a period of time prior to LPS challenge also had no effect on growth rate. As discussed in Section 4.5, the inclusion of n-3 FA's into sow diets has had variable results on piglet performance throughout the suckling phase. In the experiment outlined in Chapter 4, there were differences present in piglet growth rates based on sow dietary treatment. This is similar to findings of Rooke et al. (2001b); however, contradictory to the findings of Rooke et al. (2000) and Leonard et al. (2010a) who observed no changes in piglet growth throughout lactation following maternal consumption of fish oil. Additionally, it is possible that no dietary effects were observed in the current experiment due to a relatively short inflammatory challenge period. A single injection of LPS elicited a short response lasting approximately 24 hours, and it may be that any effects of sow dietary treatment on the growth of offspring during the inflammatory challenge require a longer period of time to be observed;

however, an LPS injection is much more potent than what pigs would typically experience in commercial practice.

Results from this experiment show that piglet body temperature was affected not only by the LPS challenge, but also by the sow dietary group from which piglets originated. Piglets originating from sows consuming the 1:1P diet had elevated body temperatures regardless of which challenge group they were assigned to relative to those from the 9:1P and 5:1P treatments. Piglets from the control diet group and 5:1F group had intermediate temperatures. This contradicts the findings of Korver and Klasing (1997), who observed that chicks fed a corn based diet had increased body temperature regardless of inflammatory challenge relative to those fed a fish oil diet. Piglets from the 1:1P treatment also had a greater febrile response to the LPS challenge, with a higher peak temperature and a longer time to return to baseline. It appears that reducing the n-6:n-3 ratio below a certain point may lead to elevated body temperatures in pigs; however, these piglets had a greater febrile response when challenged. Whether this elevated response is beneficial or potentially detrimental to the piglets needs to be examined, as it is possible that this over-stimulation of the immune system may increase production losses on farms where chronic immune challenges are presented to the animals.

Touchette et al. (2002) conducted a study looking at the effects of dietary spray-dried plasma on the immune responses of weaned piglets when challenged with LPS. Although spray-dried plasma improves animal performance at weaning, they observed an increased inflammatory response characterized by greater spikes in pro-inflammatory cytokines when pigs were challenged with LPS relative to those animals not receiving spray-dried plasma. Touchette et al. (2002) discussed the possibility of spray-dried plasma fed pigs lacking the negative feedback readiness due to reductions in basal pro-inflammatory cytokine mRNA, which caused them to have a greater spike during a strong inflammatory challenge. Although no data is available on the levels of anti-inflammatory cytokines or cytokine mRNA in the current trial, it may be possible that as the pigs were raised on diets containing more ‘anti-inflammatory properties’, the negative feedback cycle was dampened. If this is the case, these animals may do better than others when under commercial conditions, but when challenged with a strong stimulus such as an LPS dose, they spike a greater response. This may be a positive effect, in that the animals are able to cope better when a strong immune challenge presents itself, or it may have negative implications, in that animals stressed around the time of weaning mount a stronger cytokine and febrile response,

when it is not actually required. Overall, it is possible that animals raised on diets containing reduced n-6:n-3 ratios may be able to handle minor stress or pathogen challenges better, and may also be able to elicit stronger inflammatory responses when challenged with greater pathogenic loads. Further work is required to determine if this stronger response is beneficial to the animals, or a hindrance.

All four cytokines measured (IL-1β, IL-6, IL-8 and TNFα) were unaffected by diet at baseline, and thus cannot be used to explain the presence of elevated body temperatures within the 1:1P group. It is possible that other parameters of the immune system were elevated at baseline; however, they were not measured in this trial. As expected, pro-inflammatory cytokines were elevated in LPS challenged pigs, as has been reported in many studies (Carroll et al., 2003; Korver and Klasing, 1997; Webel et al., 1997). The greatest response to LPS occurred at the 2 hour time point, and most measures had returned to normal by hour 6.

No diet by challenge interactions were present for any of the measured cytokines. However, there was high variability among piglets for plasma cytokine measures. Further studies should include more than five piglets per treatment for these measures in order to reduce variability and obtain more confidence in the statistical results. An n of 5 was chosen for cytokine analysis in the current study based on the results of previous studies; however variability between pigs in the experimental herd appears to be greater than was expected. Pigs originating from sows consuming the 1:1P diet, along with having elevated febrile responses, had numerically increased IL-8 responses when challenged with LPS, and this warrants further investigation.

Typically, studies involving an LPS inflammatory challenge use blood urea nitrogen values as an indicator of muscle catabolism. The onset of an acute inflammatory reaction will partition nutrients away from growth and towards the inflammation pathway (Tizard, 2009). When the body begins to degrade muscle, elevated concentrations of BUN will be present and circulating. Webel et al. (1997) showed a clear response pattern of BUN when pigs were injected with LPS, with levels increasing up to 12 hours post LPS injection. In the current study, piglets receiving the LPS injection had greater BUN values overall; however, the time response was opposite to what Webel et al. (1997) observed. Blood urea nitrogen concentrations were highest at time 0, and decreased throughout the 12 hour measurement period for LPS and saline injected pigs. In the present trial, ADG was reduced for piglets receiving the LPS treatment, but negative

values were not observed, indicating that the short term challenge period may not have been adequate to induce a period of net muscle degradation, and ADFI also remained positive.

Overall, an inflammatory response was observed in all piglets injected with LPS relative to the saline controls. In addition, reducing the n-6:n-3 FA ratio in sow diets altered febrile responses of their offspring post-weaning; however, it remains to be determined whether this is a beneficial or detrimental response.

6.6 Conclusions

Feeding programs for sows can affect how their offspring respond to the inflammatory challenges which are presented at weaning. Altering the n-6:n-3 FA ratio in sow diets altered febrile and cytokine responses of their offspring when challenged with LPS post-weaning. We expected to see reduced inflammatory responses as the n-6:n-3 ratio decreased; however, piglets raised by sows consuming a plant based FA ratio of 1:1 had elevated rectal temperatures regardless of LPS challenge. Additionally, these piglets also had increased febrile responses when challenged with LPS compared to piglets raised by sows consuming the other diets.

Further experiments will help determine the energetic costs of these inflammatory responses on the piglets raised on different FA ratio diets, and help explain if the observed responses improve the animals ability to fight off an immune response, or if it is a hindrance. Additionally, to maximize any potential benefits of including n-3 FA's into pig diets aimed at alleviating the inflammatory responses occurring through the stressful weaning period, it would be best to feed the desired diets to sows. This would ensure that piglets obtain the n-3 FA's prior to weaning through their mother's milk.

7 GENERAL DISCUSSION & CONCLUSIONS

The current project was designed to test the overall hypothesis that reducing the n-6 to n-3 FA ratio in sow diets would improve sow reproductive performance (characterized by increased piglets born and weaned, and improved piglet growth) and would lessen the inflammatory responses of their offspring post-weaning. To test this hypothesis, the project was divided into three experiments with varied objectives. The first experiment aimed to characterize the effects of altering sow dietary FA profiles on blood, colostrum and milk FA profiles, IgA and IgG concentrations, and on animal performance throughout lactation. The objectives of experiment two were to determine if changing the sows FA profile intake would affect backfat loss, milk production and piglet growth throughout lactation, and on body fat mobilization. In experiment three, the objectives were to determine if piglets raised by sows consuming varied FA ratios would have reduced inflammatory responses post-weaning.

The results of these experiments provide information useful to the swine industry to adopt feeding strategies for sows which include n-3 FA's. Very little information was available on dietary inclusion of n-3 FA's for sows, especially in relation to dietary n-6 content. The majority of previous research studied n-3 inclusion at an added amount regardless of dietary n-6, and focused mainly on fish sources of n-3 (Cools et al., 2011; Fritsche et al., 1993; Leonard et al., 2010a; Rooke et al., 1998; Smits et al., 2011).

Feeding n-3 FA's as a ratio relative to n-6's or alternatively as an absolute amount regardless of n-6 is a controversial topic in the literature. As mentioned above, in most studies examining n-3 inclusion in swine diets, a fish based n-3 source was included into rations regardless of the n-6 content. Typically, in commercial production, a corn based basal diet will be used, which means that despite n-3 supplementation, n-6 content will be relatively higher. The metabolic pathways using these FA's are competitive, thus, the amount of n-6 in the diet relative to n-3 is important (Lands, 1992). The enzymes using the FA's as a substrate have greater affinity for n-3 FA's than n-6's, but if there is 20 times more n-6 in the diet, this competitiveness is irrelevant and the n-6's will be utilized more (Palmquist, 2009). Although the majority of this competition is within the elongase and desaturase pathway, competition between the n-3's and n-6's also occurs in the formation of the eicosanoids, and thus the ratio should also be important when feeding long chain PUFA's derived from fish sources. If 10 to 20 times more ArA is

present than EPA, a greater quantity of n-6 based eicosanoids will be formed (Calder, 1998). This led to the project hypothesis that the dietary ratio of the n-3 and n-6 FA's will be important, and that reducing the n-6:n-3 ratio will improve sow performance, reproduction and health, all of which are important to maximize animal well being, and subsequently producer profits. Typical swine dietary ratios are often greater than 20:1 n-6:n-3, and thus in order to see potential benefits of n-3 inclusion (especially plant based sources), it may be essential to reduce the dietary ratio.

Omega-3 and 6 FA's are essential to life. They are involved in many different metabolic pathways within the body, and play major roles in reproduction and immunology (Lands, 1992). In general, the n-3 FA's are considered to be anti-inflammatory whereas the n-6's are deemed pro-inflammatory (Calder, 2001). Additionally, many of the hormones synthesized from n-6's are highly active, and the n-3 counterparts are considered less biologically active. The hormones involved in these processes are formed from the 20 carbon n-6 (ArA) or n-3 (EPA). Eicosapentaenoic acid concentration in the body can be increased by direct consumption since it is found in many fish products, as well as algae, or, EPA can be synthesized within the body provided that the 18 carbon n-3 FA is present. When ALA is provided from the diet, it can undergo a series of desaturation and elongations to form EPA (Gurr et al., 2002). As mentioned above, the enzymes involved in these processes are competitive, and have higher affinity for n-3's. The rate of conversion of ALA into EPA is low however, and variable among species (Martinez-Ramirez et al., 2008). Few estimates of this conversion efficiency are available for swine. The dietary ratio may again be important, by reducing competition for these enzymes and increasing conversion of ALA into EPA.

Fish based sources of n-3's are expensive and stocks may not be sustainable. It is for this reason that the current project focused mainly on the inclusion of plant based ALA found in flaxseed and its related products, and how to best feed it relative to n-6's to elicit some of the benefits previously observed on sow and piglet performance when fish based n-3's were consumed. Non-marine sources of long chain PUFA's must be sought out and feeding strategies developed in order to maximize potential usage within the hog industry. Improving endogenous conversion of ALA into its longer chain counterparts without increasing the levels of ArA is key to increasing the adoption of n-3 FA's in sow diets.

Previous literature has shown that inclusion of fish based n-3's into sow rations, although variable across studies, can lead to improved litter size, increased piglet growth during lactation,

reduced pre-weaning mortality, increased IgG concentrations and increased embryo survival (Mateo et al., 2009; Rooke et al., 2001a; Rooke et al., 2001b; Webel et al., 2004). The current project showed improvements to litter performance (growth), sow feed intakes and body condition when sows were fed flax based n-3's relative to sows fed fish based n-3's, and pigs on the flax based n-3 diets performed as well as the control pigs. In terms of inflammatory responses, piglets had increased body temperatures and febrile responses to an LPS challenge when the ratio was dropped to 1:1. In the current study, no effects on IgG concentration, litter size or pre-weaning mortality were observed. The estimated conversion of ALA into EPA increased within the animals as the dietary n-6:n-3 ratio decreased.

Although performance was similar between the plant based n-6:n-3 ratio diets and the control diet, we did observe some negative effects when the ratio became too low. In the 3rd reproductive cycle of the trial, sows consuming a plant based ratio of 1:1 ate less feed than those consuming the control and 5:1P diets and had greater amounts of body fat throughout lactation. Those sows also mobilized more body fat in response to an epinephrine challenge compared to the 9:1P group. Additionally, piglets raised by sows consuming a 1:1 diet reacted more strongly to an LPS inflammatory challenge, as evidenced by greater cytokine peaks and febrile responses, when compared with the other piglets. This may become a concern for piglets at the time of weaning, as this over-production of inflammatory molecules may be unnecessary and energetically costly to the animal, but further work is required to confirm this.

Overall, the findings of this study show that if producers include plant based n-3 FA's into sow diets, it is important to account for the ratio of n-3's relative to n-6's to avoid potential negative effects. This study has shown that a ratio approximating 5:1 n-6:n-3 was adequate, and a ratio approaching 1:1 had negative consequences. Intermediate ratios should be investigated in future trials. A summary of significant findings is presented in Table 7.1.

The study also showed that maternal feeding can affect piglet inflammatory responses post-weaning, and thus is a potential strategy for reducing stress induced inflammatory responses post-weaning. In the current study, sows consuming the control and plant based diets performed better than those consuming the 5:1 fish based diet over time, evidenced by reduced birth and weaning weights of piglets born to 5:1F sows. One possible reason for this was a reduction in feed intake for sows consuming the fish based diet. The fish based diet had a higher fat content

when compared to the other diets, which may have led to reduced feed intakes relative to the other diets.

This project provides new insight into feeding strategies for n-3 FA's for sows and piglets. The results show there is potential for inclusion of plant based n-3 FA's into sow rations; however, there were limitations with this study that should be addressed in future research. This study covered only a small set of ratios for testing, namely 9:1, 5:1 and 1:1 n-6:n-3. Many sow rations in Western Canada are wheat/barley based, which contain a ratio close to 9:1. In the future, it would be beneficial to include a diet containing a 20:1 ratio to approximate a typical corn based production diet, in order to determine the benefit of n-6:n-3 ratio reduction from such a high ratio, or if performance results would be comparable. Also, since it appears that there may be some negative effects with an extremely low ratio (1:1), it may be warranted to test a ratio(s) between 5:1 and 1:1 in order to better assess the 'optimal' ratio for sows.

Additionally, this study has shown that one must be careful when utilizing reported values for FA profiles of oil sources. The fish based diet was formulated to have a dietary ratio of 1:1; however, when analyzed, its ratio was much closer to 5:1 due to an extremely high ArA content. Although the source of fish oil was reportedly 99% pure herring oil, the FA profile varied considerably from what is reported in the literature (NRC, 1998), and the oil should have been tested prior to use.

As in other studies, the design of these studies did not overcome the confounding issue of ratio of the FA's versus the amount. In order to modify the dietary ratio of the FA's, the amounts of at least one FA must also change. Future studies should be designed to allow for stronger statistical comparisons between the changes in ratios vs. the changes in specific FA amounts. Nonetheless, when individual FA amounts were regressed based on performance characteristics, no relationships were observed (Appendix B), indicating that ratio is more important than the actual amount of n-3 intake when evaluating plant based sources.

Table 7.1: Summary of significant findings for each experiment presented in Chapters 4, 5 and 6

Parameter	Response	P Value
Chapter 4 – Periods 1 and 2		
Piglets Born Total or Alive	No effect	> 0.10
Avg. Piglet Birth Weight	Tendency to decrease with reduced n-6:n-3 ratio	Period 1 – 0.10 Period 2 – 0.05
Number Weaned	No effect in P1, In P2 highest for 9:1P diet, intermediate for control and 5:1P diets, lowest for 1:1P and 5:1F diets	Period 1 – 0.38 Period 2 – 0.04
Avg. Piglet Wean Weight	Greatest for 9:1P and 5:1P diets, intermediate for control and 1:1P diets, lowest for 5:1F diet	Period 1 – 0.02 Period 2 – 0.04
Piglet Avg. Daily Gain	Highest for control and 5:1P diets, intermediate for 9:1P and 1:1P diets, lowest for 5:1F diet	Period 2 – 0.04
Sow Feed Intake During Lactation	No difference between control, 9:1P, 5:1P and 1:1P diets, reduced for 5:1F diet	Period 2 – 0.04
IgA and IgG Status	No effect	> 0.10
Conversion of ALA into EPA in piglets	Increased as n-6:n-3 ratio decreased in sow diets	0.01
Chapter 5 – Period 3		
Estimated Milk Production or Chemical Composition	No effect	> 0.10
Piglets Born Alive, Birth and Weaning Weights	No effect	> 0.10
Sow Feed Intake During Lactation	Highest for control and 5:1P diets, intermediate for 9:1P and 1:1P diets, lowest for 5:1 F diet	0.05
Sow Backfat Thickness	Highest in 5:1P and 1:1P fed sows	< 0.01
Baseline Plasma NEFA and Glycerol	Numerically higher in 1:1P relative to 9:1P sows	0.16
Plasma NEFA and Glycerol in response to epinephrine challenge	Numerical Increase in 9:1P relative to 1:1P sows	0.15
Plasma Leptin Concentration	Elevated in 1:1P relative to 9:1P fed sows	0.07
Chapter 6 – LPS Inflammatory Challenge		
Febrile Responses to LPS	Increased for all LPS injected pigs; Dietary effect present with 1:1P raised pigs spiking greater fevers	< 0.05
Cytokine Responses to LPS	Increased for all LPS injected pigs; 1:1P and 5:1F raised pigs had numerically greater IL-8 responses post-challenge	LPS – < 0.06 Diet x Challenge for IL-8 – 0.18

The implications of the results from the LPS challenge study are difficult to interpret. Piglets born to sows consuming a 1:1 ratio had increased febrile responses to the LPS challenge than other pigs. It is unclear if this response is a benefit or a detriment. It is possible that these pigs may generate a strong inflammatory response to mild pathogens or stress when not under a direct disease/stress challenge. This is energetically expensive for the animals and thus inefficient or, it may mean that these animals can fight off an infection faster when they experience a large immune stimulus. Future studies are required to determine the metabolic cost of this inflammatory response to the animal, and how it affects protein turnover in the body. The additional information would allow a more complete interpretation of the results of the current LPS challenge study. Based on the conclusions of a study by Touchette et al. (2002), it is possible that piglets raised on diets with more 'anti-inflammatory' properties have a dampened negative feedback response within the inflammatory process, which may allow them to perform better under standard commercial conditions but may allow for a stronger inflammatory response when challenged with a large pathogen load.

Within these experiments, there were several situations where large numeric differences were observed in measured parameters; however, statistical significance was not achieved. An example is the epinephrine challenge described in Chapter 5, where doubling of differences did not achieve statistical significance (fed NEFA and glycerol concentrations in Table 5.5; glucose, NEFA and glycerol niAUC and peak values in Table 5.6). Variability among animals was extremely high for these challenges, making interpretation of the results difficult. The number of animals used was based on previous reports; however, a much greater number of animals would be required to reduce animal variability to improve statistical power. Based on the variability observed in our trial, we would need several thousand animals to observe significance, thus future experiments should be designed to closely examine the source of this variation. Standard errors were also high for the measurements of cytokines in the LPS challenge (Chapter 6). Although cytokine assays are improving with time, there are still several challenges which may have affected laboratory analysis such as sample freeze/thaw cycles, contamination of plasma, etc. These potential errors combined with large animal variability affect the interpretation of results presented here.

To date, very little information regarding n-3 based feeding strategies for sows is available. These essential FA's have great potential to improve animal performance and health,

not just for sows but for all stages of swine production, and should be a focus for nutritionists to develop accurate requirement values and optimal feeding programs, which is important also due to the high cost of feeding n-3 FA's. Swine nutritionists have spent the majority of time researching ways to improve growing pig performance and carcass characteristics, with relatively little information being generated on sow nutrition requirements. This study opens up a variety of possible future research areas focusing on sow nutrition as a means to improve production profits, animal health, animal welfare and overall performance.

In summary, it is important to consider the n-6 FA concentration of the diet when incorporating n-3 FA's into sow diets. Sows consuming a plant based 1:1 ratio or a fish based 5:1 consumed less feed than sows on the other diets. Additionally, sows consuming a 1:1 ratio were in a state of negative energy balance relative to those consuming a 9:1 based diet throughout early lactation, which can in turn lead to reduced reproductive lifespan and increased herd turnover (Boyd et al., 2000), thus increasing the overall cost of production. Piglets born to sows consuming a fish based 5:1 ratio were lighter at birth and weaning relative to the other treatment groups. Results from these studies also show that when the dietary n-6:n-3 ratio decreases, conversion of ALA into EPA increases within the body.

In conclusion, there is potential for including plant based n-3 FA's into gestation and lactation rations for sows when their ratio relative to n-6 FA's is considered. The long term feeding of decreased n-6:n-3 ratio diets (down to a 5:1 ratio) did not have negative impacts on sow or piglet performances relative to a tallow based control diet, but did improve the conversion of ALA into EPA, and increase the transfer of long chain n-3's to piglets through milk. Overall, feeding a plant based n-3 FA to sows, when fed at an optimal ratio relative to n-6 inclusion, can impact sow reproduction, piglet performance and health. We hypothesized that reducing dietary n-6:n-3 ratios in sows will improve sow reproductive performance. We conclude that there are advantages to reducing the ratio from the 9:1 resulting from wheat/barley based diets, however, detrimental effects are observed if the n-6:n-3 ratio in sow diets approaches 1:1. Reducing the n-6 to n-3 FA ratio in sow diets did not affect IgG or IgA status, thus we reject the hypothesis that reducing the FA ratio would improve the passive immune status of the piglets post-farrowing. There was evidence, however, that sow dietary FA profiles will mediate inflammatory responses in weaned piglets.

8 IMPLICATIONS

The use of n-3 FA's is of growing interest to the swine industry, mainly due to their known health benefits to not only the animals, but also to consumers of the pork. Typically, the n-3's included in swine rations are EPA and DHA, derived from fish, as they are more biologically active compared with the 18-carbon plant based n-3's. This study has shown that plant based n-3's can be incorporated into swine diets in a safe and effective manner, and are able to alter biological responses of sows and their offspring. It is important to ensure that the ratio relative to n-6 FA's is considered in the feed formulation.

Results from this study provide valuable information to the swine and feed industries, potentially opening up a new area of feeding strategies for sows and their offspring. Additionally, feeding plant based n-3's to sows may provide a new outlet for the flax industry. Canada is the leading producer of flaxseed in the world, producing approximately 40% of the world crop. The majority of this is produced in the Prairie Provinces of Alberta and Saskatchewan; however, over 55% of the Canadian supply is typically shipped overseas to countries such as Belgium for crushing (StatCan, 2011). Providing new, local, outlets for the by-products may assist with the development of a Canadian flax crushing industry and improve profits of both the swine and flax industries. Additionally, high-oil flax by products such as the FSM produced by Vandeputte s. a. may have a higher value than other meal products, and can be incorporated into sow diets as a source of plant based n-3 FA's.

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10 APPENDIX A

The following diagrams and pictures outline the layout of the breeding, gestation and farrowing sow housing facilities described in detail in Chapter 4.

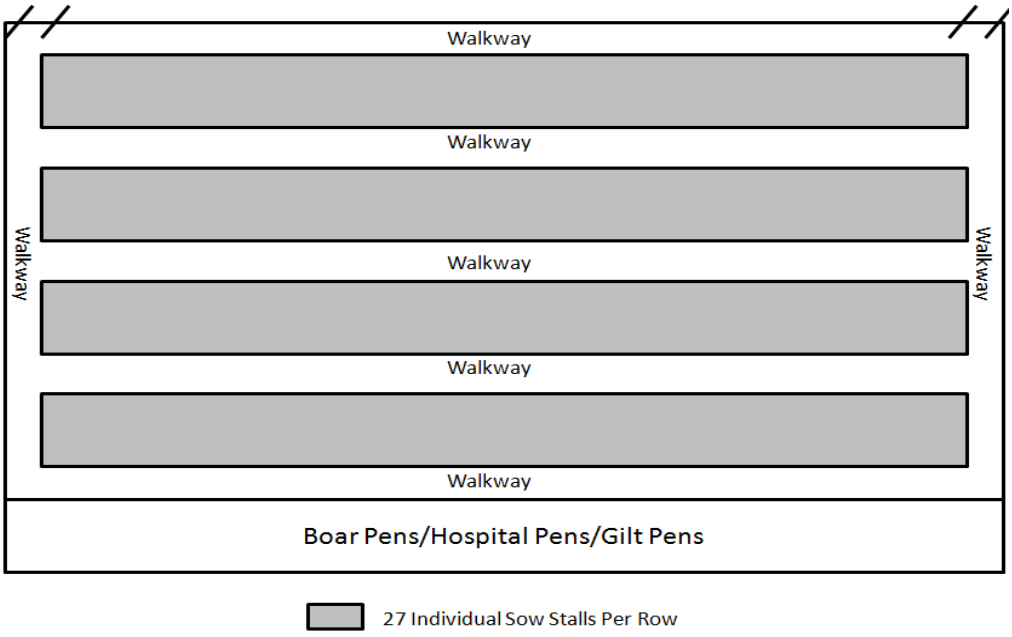

Figure A.1: Sow breeding facility layout

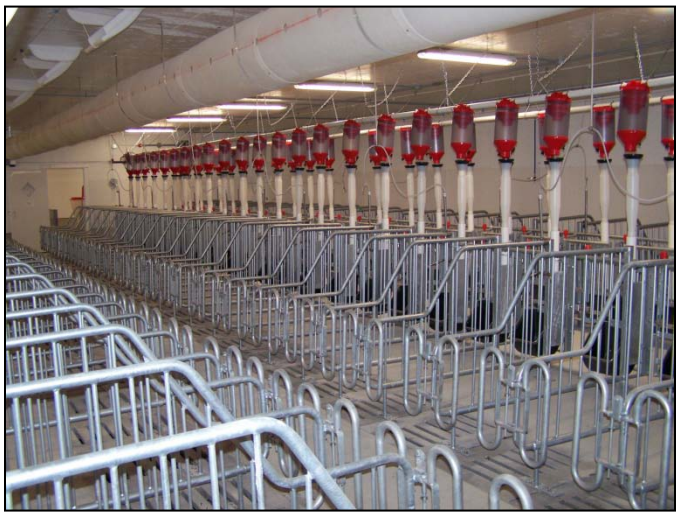

Figure A.2: Row of stalls within the breeding facility

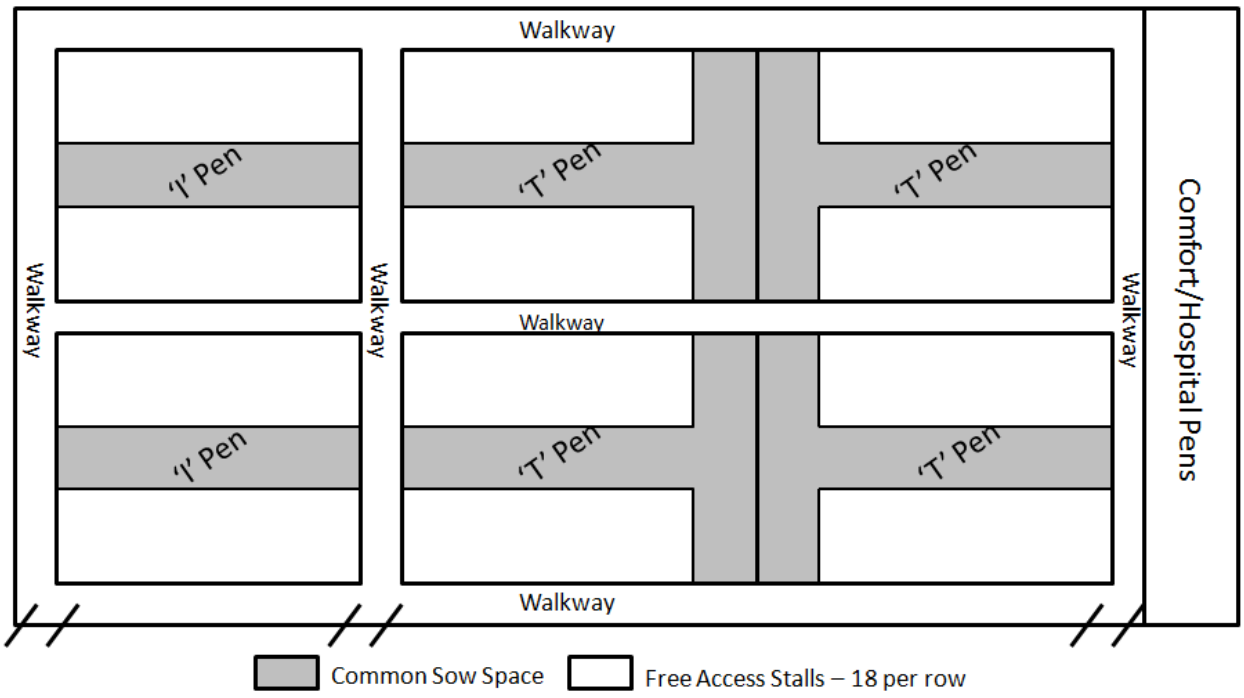

Figure A.3: Layout of group housed gestation facility

Figure A.4: Design of a 'T' shaped pen in the gestation facility. The 'I' pens were similar but did not contain the additional solid floor loafing area.

Figure A.5: Gestation free-access stall design

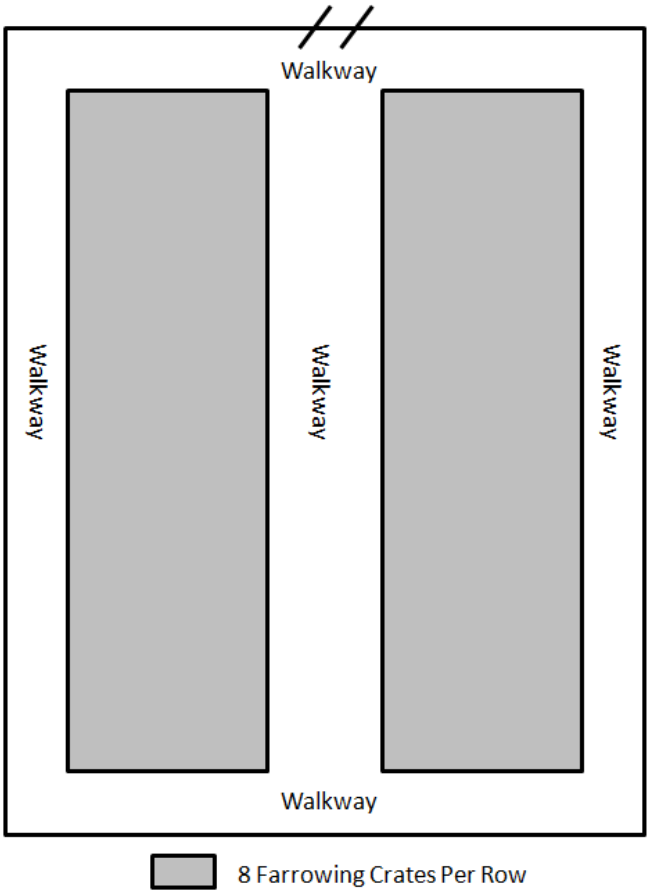

Figure A.4: Farrowing room layout

Figure A.5: Farrowing crate design

11 APPENDIX B

One major confounding factor present in most studies involving the dietary n-6:n-3 FA ratio is the fact that it is extremely hard to separate the effects of altering the dietary ratio from that of the dietary amount of the individual FA's. When the ratio is altered, at least one FA must change in amount also. In order to determine if the amount of FA intake was more important in this trial, a series of regressions were plotted looking at individual FA intake (g FA/d during lactation) on production parameters recorded throughout the trial.

The following series of figures show that regression coefficients were extremely low when looking at individual FA intake effects on sow and piglet performance, and from that combined with all previously described data, we concluded that the ratio of n-3 FA's in relation to n-6 FA's is the more important parameter to consider when formulating sow rations to contain n-3 FA's. A selection of graphs are shown to illustrate the point; however, similar results were observed for the effects of ALA, EPA, DHA, LA, ArA, total n-3 and total n-6 intake (g/d) on all production parameters discussed in Chapters 4 and 5.

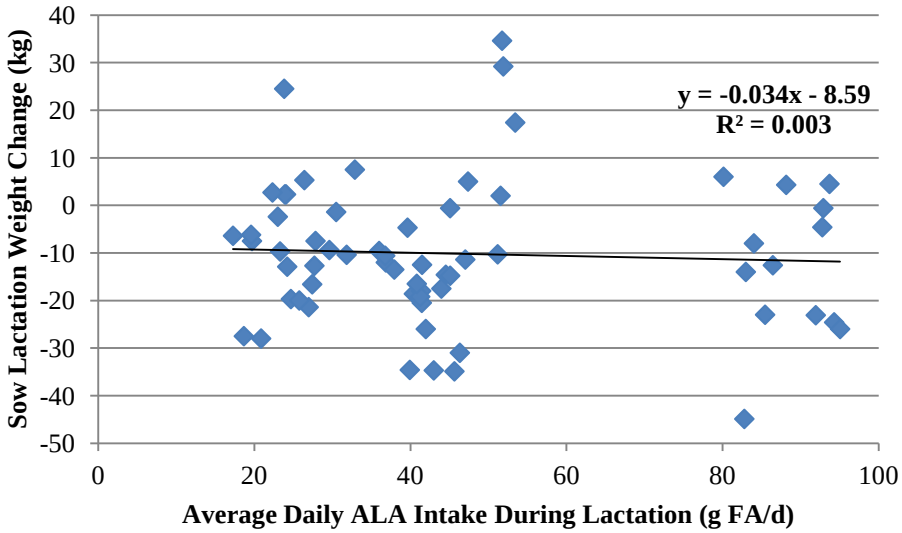

Figure B.1: Sow daily α-linolenic acid (ALA) intake throughout lactation vs. sow body weight change for a 26 ± 2 d lactation period

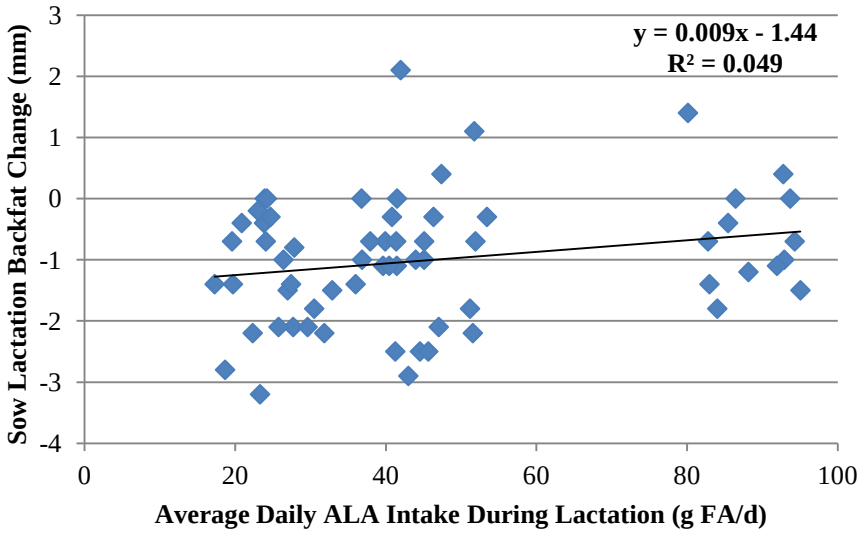

Figure B.2: Sow daily α-linolenic acid (ALA) intake throughout lactation vs. sow backfat change for a 26 ± 2 d lactation period

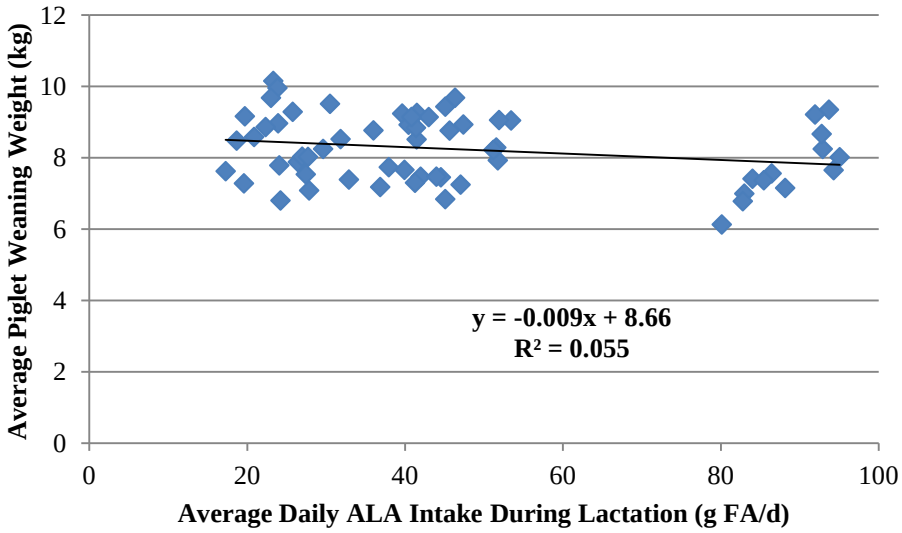

Figure B.3: Sow daily α-linolenic acid (ALA) intake throughout lactation vs. average piglet weaning weight for a 26 ± 2 d lactation period

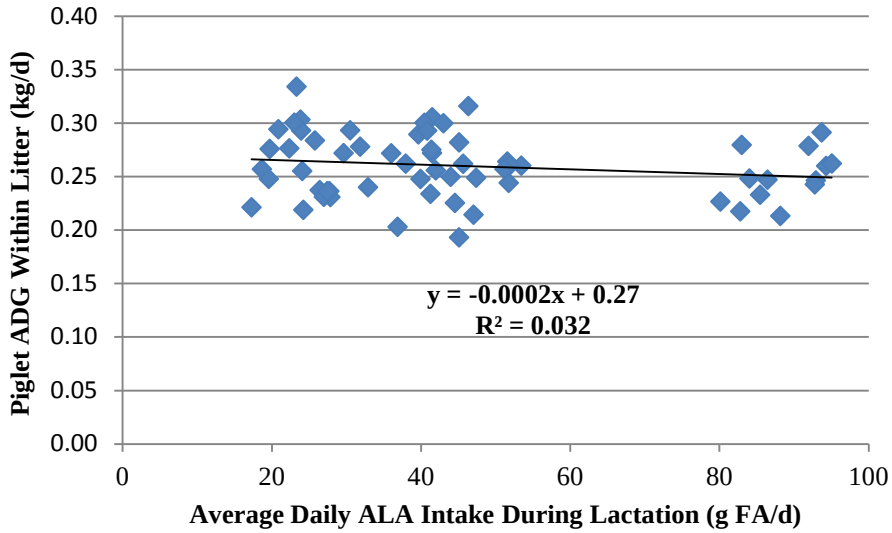

Figure B.4: Sow daily α-linolenic acid (ALA) intake throughout lactation vs. piglet average daily gain within litter for a 26 ± 2 d lactation period

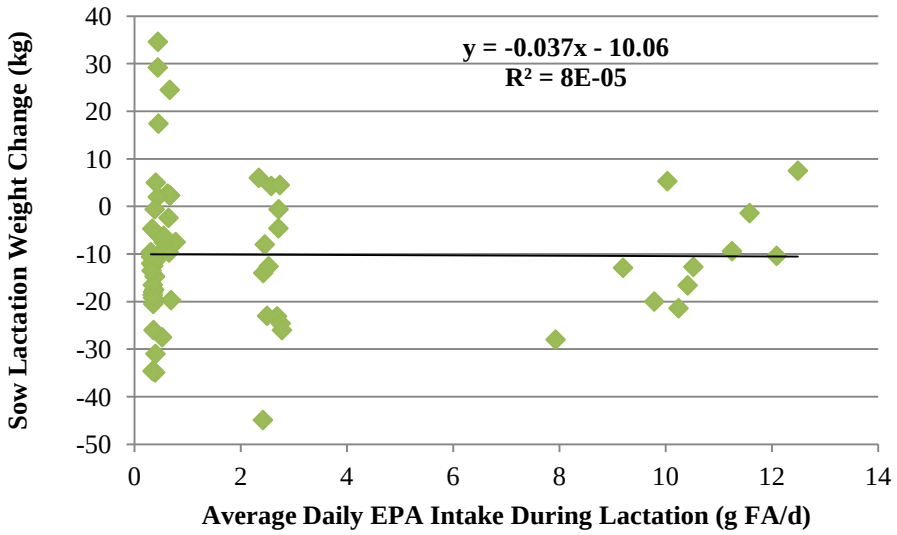

Figure B.5: Sow daily eicosapentaenoic acid (EPA) intake throughout lactation vs. sow body weight change for a 26 ± 2 d lactation period

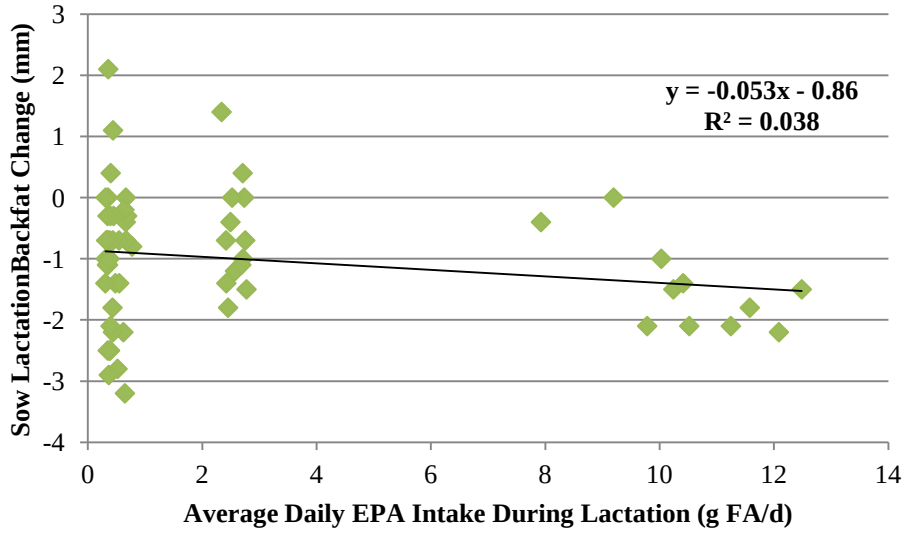

Figure B.6: Sow daily eicosapentaenoic acid (EPA) intake throughout lactation vs. sow backfat change for a 26 ± 2 d lactation period

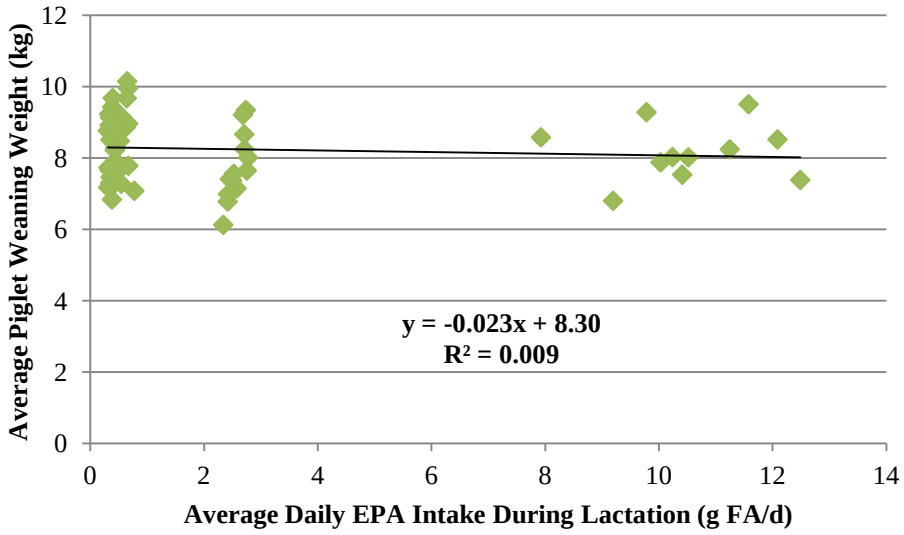

Figure B.7: Sow daily eicosapentaenoic acid (EPA) intake throughout lactation vs. average daily piglet weaning weight for a 26 ± 2 d lactation period

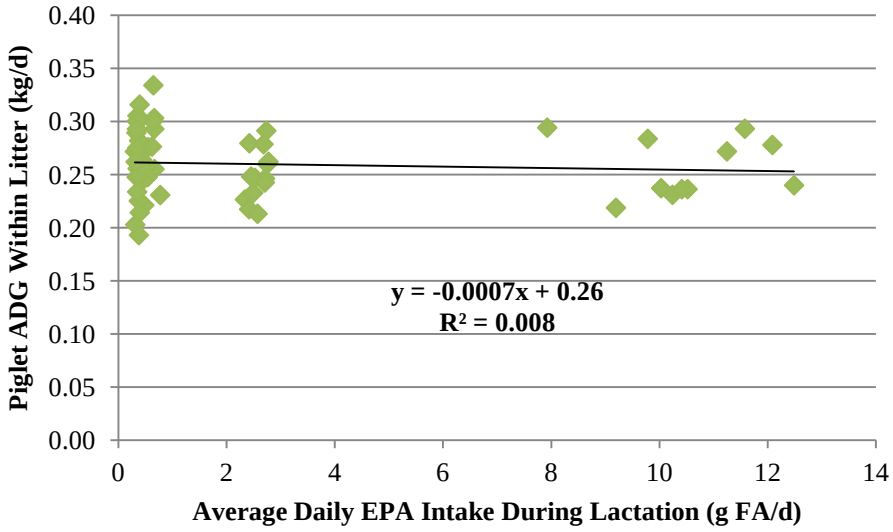

Figure B.8: Sow daily eicosapentaenoic acid (EPA) intake throughout lactation vs. piglet average daily gain within litter for a 26 ± 2 d lactation period

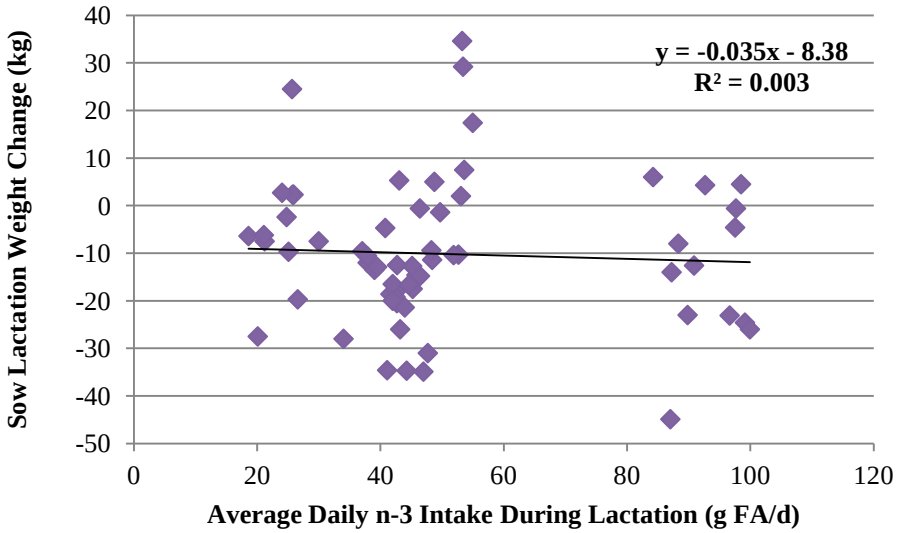

Figure B.9: Sow daily omega-3 (n-3) intake throughout lactation vs. sow body weight change for a 26 ± 2 d lactation period

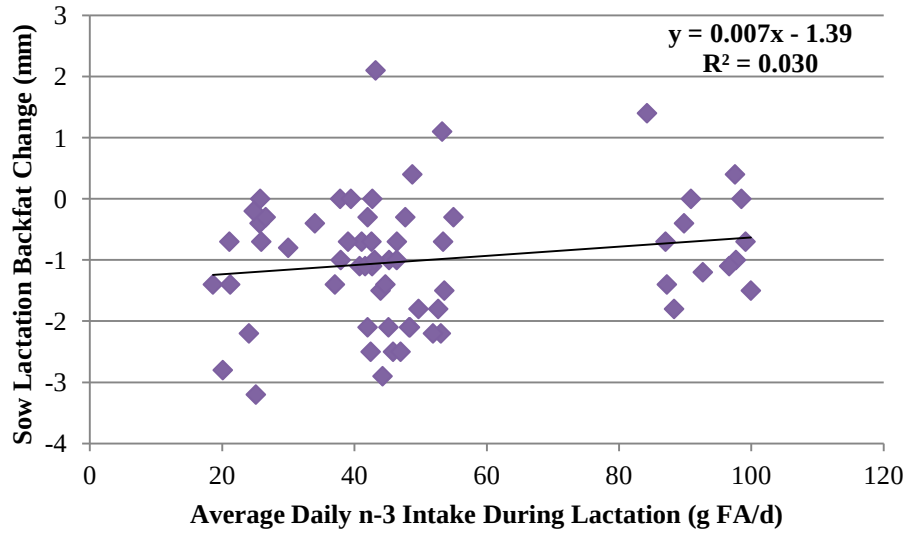

Figure B.10: Sow daily omega-3 (n-3) intake throughout lactation vs. sow backfat change for a 26 ± 2 d lactation period

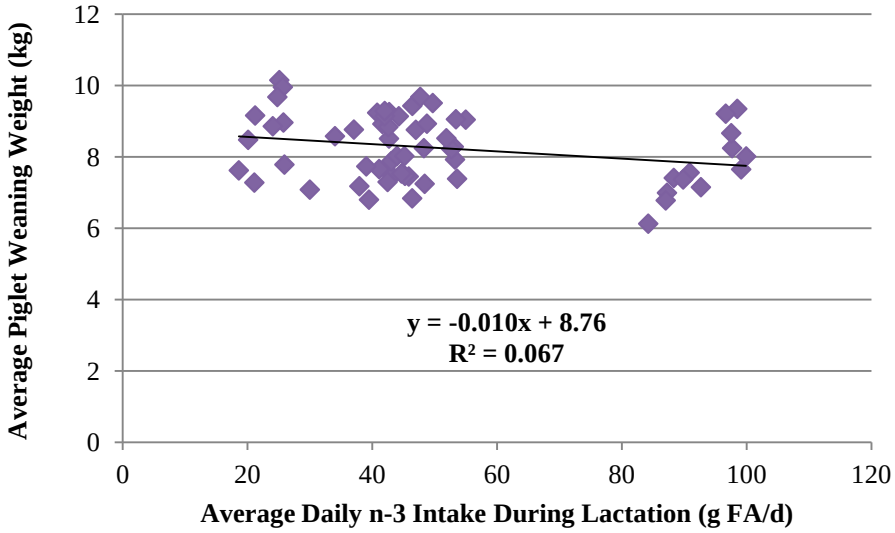

Figure B.11: Sow daily omega-3 (n-3) intake throughout lactation vs. average daily piglet weaning weight for a 26 ± 2 d lactation period

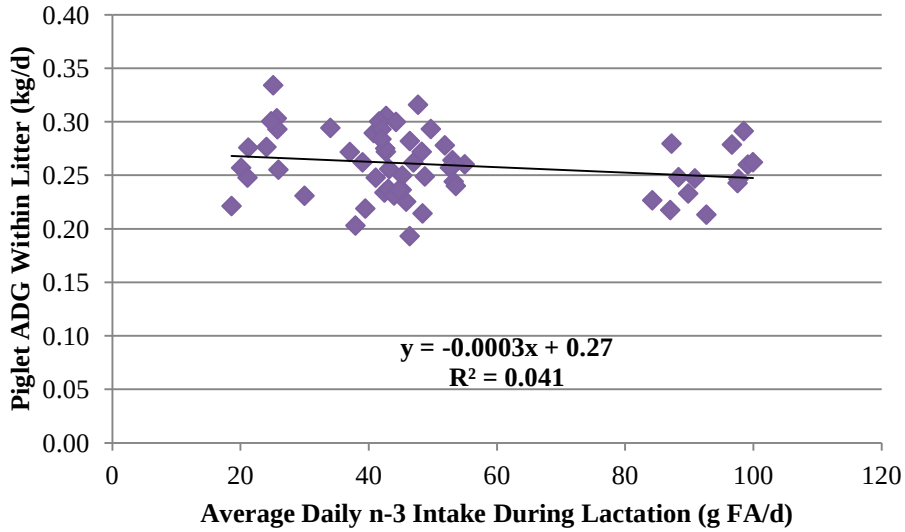

Figure B.12: Sow daily omega-3 (n-3) intake throughout lactation vs. piglet average daily gain within litter for a 26 ± 2 d lactation period